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Basic Study

Integrative transcriptomic and proteomic analysis reveals that SERPING1 inhibits neuronal proliferation *via* the CaMKII-CREB-BDNF pathway in schizophrenia

The pathogenic mechanism of schizophrenia

Feng Li, Xing Ren, Jia-Xiu Liu, Tian-Dao Wang, Bi Wang, Xiao-Bin Wei

Abstract

BACKGROUND

Schizophrenia (SZ) is a prevalent chronic brain disorder characterized by an unclear etiology and pathogenesis. Although no widely accepted endophenotype exists, peripheral blood mononuclear cells (PBMCs) offer a viable model for studying intracellular changes associated with SZ.

AIM

This study aims to preliminarily explore the pathogenic mechanisms and identify effective biomarkers for SZ.

METHODS

An integrative transcriptomics and proteomics analysis of PBMCs from SZ patients was conducted to identify differentially expressed genes (DEGs) and differentially expressed proteins (DEPs), and to predict potential signaling pathways involved in the disease. Western blotting (WB) and RT-qPCR validated the DEGs and DEPs. Rat hippocampal CA1 neurons were transfected *in vitro* with RNAi-lentivirus, and target gene expression was validated by RT-qPCR. Neuronal conduction proteins caMKII, CREB, and BDNF were assessed by WB. Flow cytometry was used to detect cell apoptosis, and cell proliferation or viability was measured using the Cell Counting Kit-8.

RESULTS

Integration of transcriptomics and proteomics revealed 6,079 co-expressed genes, including 25 DEGs between the SZ group and healthy controls, with significantly upregulated HP, LTF, and SERPING1. KEGG enrichment analysis indicated that neuroactive ligand-receptor-related pathways might be crucial in disease progression. Initial validation with clinical samples showed significantly higher protein and mRNA levels of HP, LTF, and SERPING1 in the SZ group compared to healthy controls. WB

validation with all clinical samples confirmed a significant increase in SERPING1 expression. In hippocampal CA1 neurons transfected with lentivirus, SERPING1 expression was significantly reduced, while caMKII, CREB, and BDNF expression levels increased, along with decreased apoptosis and increased cell viability.

CONCLUSION

SERPING1 may inhibit neural cell proliferation in SZ patients through the CaMKII-CREB-BDNF pathway.

Key Words: Transcriptomics; Proteomics; Schizophrenia; SERPING1; Pathogenesis

Core Tip: The strength of this study lies in the integration of transcriptomics and proteomics data from the same sample source (peripheral blood mononuclear cells) to better observe mRNA-protein inconsistencies and mRNA-protein correlations, thus explaining the biology of schizophrenia in a holistic manner. Proteomics and transcriptomics correlation analyses provide a panoramic view of the expression profile of schizophrenia, enabling complementarity and integration at the transcriptional and protein levels. Again, this study is one of the few to investigate the role of the SERPING1 gene in neural cell proliferation and apoptosis.

INTRODUCTION

Schizophrenia (SZ) is a severe mental disorder marked ²⁸ by positive symptoms such as hallucinations, delusions, and speech disorders and negative symptoms including hypobulia, anhedonia, and social withdrawal, along with cognitive impairment. The disorder progresses through at least three stages: Prodromal (pre-psychotic), psychotic, and chronic[1, 2]. Diagnosis primarily relies on clinical symptoms, lacking diagnostic auxiliary tests or biomarkers[3], complicating early diagnosis, disease stratification, treatment selection, and outcome prediction. This often results in misdiagnosis, missed diagnosis, treatment failure, or relapse. The unclear pathogenesis of SZ indicates its

association with neurodevelopmental disorders, genetic susceptibility, and cumulative developmental environmental factors[4, 5]. Inflammation, immune system changes[6, 7], and impaired brain and systemic energy metabolism[8] also correlate with SZ. Epidemiological research by Michael E. Benros *et al.*[9] reveals that severe infections and autoimmune diseases heighten SZ risk. Additionally, long-term remission of psychotic symptoms in patients on extended warfarin therapy for deep vein thrombosis suggests a close relationship between coagulation pathway abnormalities and SZ progression[10].

Genome-wide association studies (GWAS) have identified over 100 risk genes related to SZ[11], encompassing major functional gene categories such as neuronal, immune, and histone pathways[12]. Despite these findings, elucidating how these genes confer SZ risk remains complex. Current SZ research models primarily utilize post-mortem tissues, blood samples, and patient-derived induced pluripotent stem cells (iPSCs). The acquisition and preservation of post-mortem samples pose significant challenges, unlike the more accessible blood samples. iPSC models, while valuable for studying basic processes within and between neural cells, are less suitable for case-control studies due to statistical limitations and difficulties in identifying significant cell phenotypes at the individual level[13]. Blood samples offer insights into the body's immune status, with evidence suggesting that central nervous system activity in SZ influences peripheral blood gene expression[14]. Peripheral blood mononuclear cells (PBMCs), recognized for their high sensitivity and specificity as biomarkers for various diseases, are extensively used in SZ research[15, 16]. This study undertakes a preliminary exploration of SZ pathogenesis through integrative transcriptomics and proteomics analysis of PBMCs from SZ patients and related *in vitro* experiments.

MATERIALS AND METHODS

Research subjects

SZ group

This study included 150 SZ inpatients (6 for omics analysis, 150 for Western blotting [WB] detection) from Hainan Anning Hospital, enrolled between March and July 2022. Clinical data such as age, age of onset, gender, SZ subtype, family history, and head imaging were collected. The clinical information of SZ used in the omics analysis was detailed in **Table 1**. Inclusion criteria mandated a diagnosis of SZ according to the International Statistical Classification of Disease and Related Health Problems, 10th Revision (ICD-10), confirmed by two trained specialists. Exclusion criteria ruled out mental retardation or other mental disorders, neurological diseases, major physical diseases (*e.g.*, cancer, leukemia), severe infectious diseases (*e.g.*, AIDS), and pregnancy or lactation.

Healthy control group

A control group of 150 healthy controls (HC) from Haikou People's Hospital, matched for age and gender with the SZ group, was included during the same period. Inclusion criteria were: No history of mental illness or family history of mental illness, no mental retardation, no history of alcohol, drug, or substance abuse, no chronic disease history, no head trauma or surgery, and not pregnant or lactating.

Neuronal cells in the CA1 region of the hippocampus of male healthy SD rats

Informed consent

This study received approval from the Biomedical Ethics Committee of ²⁰ Haikou Hospital affiliated with Xiangya School of Medicine, Central South University (Haikou People's Hospital), under the ethics approval number 2021-(Ethics Review)-263 (**Ethical statement 1**). Additionally, it ²⁵ was approved by the Biomedical Ethics Committee of Hainan Anning Hospital, with the ethics approval number 2022-(Ethics Review)-2 (**Ethical statement 2**). Informed consent was obtained from the subjects or their guardians, with the consent form approved by the Medical Ethics Committee signed prior to the study's implementation.

Experimental methods and steps

Collection and PBMC extraction of clinical samples

Using 9 mL sodium citrate anticoagulant vacuum blood collection tubes (sodium citrate: Blood = 1: 9), fresh fasting venous whole blood was collected from the subjects. The samples were processed with lymphocyte separation solution to obtain PBMCs. An appropriate amount of PBS was added to wash the cells, the supernatant was discarded, and the PBMCs were collected and stored in a -80°C freezer.

RNA extraction and sequencing

¹ An appropriate amount of PBMCs was thoroughly ground in liquid nitrogen, transferred to a 1.5 mL centrifuge tube, and subjected to RNA extraction *via* the Trizol method. RNA concentration and purity were measured with an enzyme-labeled instrument, and qualified RNA was stored at -80°C. Using the poly-A structure at the 3' end of mRNA, magnetic beads with Oligo(dT) enriched the mRNA, which was reverse transcribed into cDNA to create a cDNA library. Samples were analyzed with the DNA 1000 assay kit (Agilent Technology, China) within a detection fragment ²⁷ size range of 25-1000 bp and a concentration range of 0.1-50 ng/μL. Qualified samples underwent sequencing on the Illumina HiSeq Xten platform. Compare the original data quality control with the reference genome, filter unqualified sequences in the original data to obtain valid data, and use HISAT2 software (<https://daehwankimlab.github.io/hisat2/>) to compare the valid data with the reference genome. Calculating gene expression levels: ²³ The FPKM value of each gene's expression in each sample can be calculated with the featureCounts software (<http://bioinf.wehi.edu.au/featureCounts/>). ⁵ The calculated read count can be used to compare the recounts between samples. Differential expression analysis: DESeq2 (<https://bioconductor.org/packages/release/bioc/html/DESeq2.html>) and edgeR software (<https://bioconductor.org/packages/release/bioc/html/edgeR.html>) were used to conduct gene differential expression analysis, and the P-value obtained from hypothesis

test was calibrated to obtain $p\text{-adj. } p\text{-adj} < 0.05$ and $|\log_2\text{foldchange}| > 1$ were set as the significance criteria of DEGs.

Quantitative analysis of data-independent acquisition (DIA) proteomics

SDT buffer was added to the PBMCs, and the lysate underwent ultrasonication. The mixture was then boiled for 15 minutes and centrifuged at 14,000 g for 40 minutes. The supernatant was quantified using a BCA protein assay kit (Bio-Rad). Equal amounts of small portions from each sample were pooled. This pooled sample was fractionated by HPRP and analyzed using LC-MS/MS (QE-HFX_DDA mode). A spectral library was constructed using Spectronaut Pulsar X software (<https://biognosys.com/resources/spectronaut-manual/>) to create the DDA library database. Each sample was then analyzed separately using LC-MS/MS (DIA mode), with qualitative and quantitative analyses performed using the DDA database.

Integrative transcriptomics and proteomics analysis

Integrative transcriptomics and proteomics analysis was conducted using R language (<https://www.r-project.org/>) and software such as Blast2 (<https://www.blast2go.com/>).

Validation of protein expression by WB in clinical samples

Total protein from PBMCs was separated by SDS-PAGE and transferred to a PVDF membrane (Merck Millipore, Bedford, MA, USA). Blocking was performed with 5% non-fat milk in PBST solution, followed by overnight incubation at 4°C with primary antibodies (anti-HP, anti-LTF, anti-SERPING1, Abcam, UK). Afterward, the membrane was incubated for 40 minutes at room temperature with secondary antibodies. Enhanced chemiluminescence imaging using an ECL kit (Beyotime, Shanghai, China) was then conducted. GAPDH served as the internal control protein, and ImageJ software facilitated grayscale analysis to determine the relative expression levels of the target proteins.

RT-qPCR detection of expression in clinical samples

Total RNA extracted from PBMCs was used as a template to synthesize cDNA according to the Thermo qPCR RT Kit manual. Real-time fluorescence quantitative PCR was then performed using TransGen Biotech® SybrGreen qPCR SuperMix. The specific primer sequences for the genes tested are listed in **Table 2**. The relative expression of target gene mRNA was calculated using the $2^{-\Delta\Delta CT}$ method (Livak method).

Rat hippocampal tissue isolation and cell culture

Male healthy SD rats, weighing 250 ± 20 g, were housed in a standard animal room at $21 \pm 2^\circ\text{C}$ and 40-70% relative humidity. The room was kept quiet, bedding was changed frequently, and the rats were fed regular rodent chow. Anesthesia was induced with 0.3% sodium pentobarbital (1 mL/100 g). Rats were perfused and fixed with physiological saline and 4% paraformaldehyde. The brain tissue was removed, and the hippocampus was isolated and stored at -80°C . The hippocampal CA1 region tissue was washed three times with D-Hanks solution, minced, and digested with 0.25% trypsin at 37°C until dispersed into cell clusters or single cells. After centrifugation at 500 rpm for 5 minutes, the supernatant was discarded, and the cells were resuspended in a complete culture medium for rat neurons. Cell density was adjusted to 2×10^6 cells/mL after counting with a hemocytometer, and cells were dispensed into culture flasks and incubated at 37°C with 5% CO_2 .

Construction of RNAi-silencing lentivirus

The SERPING1 gene sequence (GENE ID: 2182) was obtained from NCBI, and interference targets were designed accordingly, as detailed in **Table 3**. The Plko.1-EGFP-PURO vector was used for cloning, with Age I (ACCGGT) and EcoR I (GAATTC) as cloning sites. The recombinant plasmid was amplified and extracted using the OMEGA Endotoxin-Free Plasmid Mini Kit. The plasmid A260/280 ratio and concentration were measured, and the plasmid was stored at -20°C Serum-free, double-

antibody-free DMEM (1 mL) was prepared, and lentiviral packaging and expression plasmids were added, followed by the transfection reagent. The mixture was vortexed and allowed to stand at room temperature for 30 minutes to form a polymer. This polymer was added dropwise to 293T cells with fresh medium, and the cells were further cultured. The viral supernatant was collected, centrifuged, filtered, concentrated, purified, aliquoted, and stored at -80°C. Lentivirus-infected 293T cells were analyzed using flow cytometry and fluorescence microscopy to determine the lentiviral packaging titer. Results are provided in **Supplementary File 1**.

Lentiviral infection of target cells (neuronal cells in the CA1 region of the rat hippocampus)

First, 50 µl of Opti-Medium was used to dilute the si-SERPING1 Lentivirus and gently mixed by pipetting. The transfection reagent was gently inverted to mix. Then, 1.2 µl of Lipofectamine 2000 was diluted in 50 µl of Opti-Medium, gently mixed by pipetting, and allowed to stand at room temperature for 5 minutes. The diluted transfection reagent and si-SERPING1 Lentivirus solution were combined and gently mixed by pipetting, then left to stand at room temperature for 20 minutes. The transfection complexes were added to a 24-well cell culture plate, 100 µl per well, and the plate was gently rocked back and forth to mix evenly. The cell culture plate was incubated at 37°C with 5% CO₂ for 24-48 hours, with the transfection process lasting 4-6 hours.

RT-qPCR detection of SERPING1 expression in neuronal cells in the CA1 region of rat hippocampus

Lentivirus transfection was performed in the following groups: NC (negative control), si-SERPING1#1, si-SERPING1#2, and si-SERPING1#3 (three silencing targets). After 4-6 hours of transfection, RNA was extracted from the target cells, and cDNA was synthesized using the Thermo qPCR RT Kit. Real-time fluorescence quantitative PCR was conducted using TransGen Biotech® SybrGreen qPCR SuperMix. The primer sequences for the rat SERPING1 gene are as follows:

Forward primer (5'→3'): CGCCTCTCTGAGCCTGTATG

Reverse primer (5'→3'): TCAGTTCCAACACCGTCTCG

¹ The relative expression of the target gene mRNA was calculated using the $2^{-\Delta\Delta CT}$ method (Livak method).

WB detection of neuronal expression of neural-related conductance proteins caMKII, CREB, BDNF in neuronal cells of CA1 region of rat hippocampus

Lentivirus transfection was performed in the following groups: Control, negative control (NC), and si-SERPING1#1 (the one with the highest silencing efficiency among the three). After 4-6 hours of transfection, WB detection was conducted according to the specific steps outlined in section 2.2.5.

Apoptosis of target cells detected by flow cytometry

Rat hippocampal neurons in the logarithmic growth phase ¹⁷ were seeded in a 6-well culture plate at a density of 7×10^5 cells per well, with a total medium volume of 2 mL per well. Lentivirus transfection was performed in the groups: Control, negative control (NC), and si-SERPING1#1. After 4-6 hours of transfection, cells from each group were collected by centrifugation, ²¹ washed twice with pre-cooled PBS at 4°C, and resuspended in 500 µl of binding buffer, adjusting the concentration to ² 10^6 cells/mL. Then, 100 µl of the cell suspension was transferred to a 5 mL flow cytometry tube. Annexin V-APC (5 µl) was added and mixed, followed by ³ 5 µl of Propidium Iodide, mixed again, and incubated at room temperature in the dark for 15 minutes. Apoptosis was analyzed using a flow cytometer (FACS). Detailed steps are provided in the Biolegend Annexin V-FITC/PI double-staining apoptosis detection kit manual.

Proliferation of target cells detected by cell counting kit-8

²² The viability of rat hippocampal neurons was assessed using the Cell Counting Kit-8 (CCK-8, Solarbio, Beijing, China). Lentivirus transfection was performed in the following groups: Control, negative control (NC), and si-SERPING1#1. Post-

transfection, cells from each group were digested with trypsin and seeded in a 96-well plate at a density of 8000 cells per well. Once stabilized, CCK-8 assays were conducted on days 1, 2, 3, 4, and 5. At each time point, 10 μ l of CCK-8 was added to each well, and cells were incubated at 37°C in a 5% CO₂ incubator for 1-4 hours. Optical density (OD) at 450 nm was measured using a microplate reader (ELx800, BioTech, Vermont, USA). Results were recorded, and a cell growth curve was plotted with time on the x-axis and OD values on the y-axis.

Statistical analysis

Experimental data were analyzed and plotted using GraphPad Prism 9 software. Results were expressed as $\bar{x} \pm SD$. Multiple t-tests compared two groups, while one-way analysis of variance (ANOVA) compared multiple groups. Statistical data were measured by χ^2 test. A P-value less than 0.05 was considered statistically significant.

RESULTS

RNA sequencing results

A total of 3317 differentially expressed genes (DEGs) were identified, comprising 1635 upregulated and 1682 downregulated genes. The top 10 genes with the most significant statistical differences in gene expression are presented in **Figure 1a**, with their details provided in **Table 4**. Clustering analysis demonstrated that DEGs effectively differentiated SZ from HC, highlighting notable alterations in PBMC gene expression in SZ patients (**Figure 1b**).

Quantitative analysis of DIA proteomics

DIA proteomics quantitative analysis was performed on the SZ and HC groups. The overall number of identified proteins and peptides for each sample in both groups is illustrated in a bar chart in **Figure 2a**. To identify differentially expressed proteins (DEPs) between the groups, differential screening of the experimental data was conducted. Significant DEPs were screened using criteria of fold change (FC) > 1.5

4 (upregulated by more than 1.5 times or downregulated by less than 0.67 times) and a P-value < 0.05. This screening identified 204 upregulated proteins and 44 downregulated proteins, as shown in **Figure 2b**. The ten most significantly upregulated and downregulated DEPs are labeled in **Figure 2c**. Clustering analysis of DEP expression demonstrated that these proteins effectively distinguished between the SZ and HC groups, indicating significant alterations in PBMC protein expression patterns in SZ patients, as shown in **Figure 2d**.

Integrative transcriptomics and proteomics analysis

Integration of transcriptomics and proteomics identified 6079 commonly expressed genes, including 25 DEGs between the SZ and HC groups (**Figure 3a**). Among these, the significantly upregulated genes were HP, LTF, and SERPING1 (**Table 5**). Correlation analysis between significant DEGs and DEPs yielded a Spearman correlation coefficient of 0.3928, confirming the data's reliability (**Figure 3b**). Clustering analysis visually presented the expression patterns of differentially expressed mRNAs and DEPs (**Figure 3c**). Additionally, KEGG enrichment analysis of the 25 DEGs suggested that the neuroactive ligand-receptor interaction pathway may play a crucial role in the progression of SZ (**Figure 3d**). So, for the next possible mechanism verification, we selected the neuro-associated conduction protein pathway for related research.

Significantly higher expression levels of DEGs initially validated in small clinical samples

Protein expression levels of HP, LTF, and SERPING1 in PBMCs derived from clinical samples were detected using WB, which exhibited significant upregulation in the combined transcriptomics and proteomics analysis. HP, LTF, and SERPING1 proteins exhibited significantly higher expression in the SZ group compared to the HC group (**Figure 4**). Additionally, RT-qPCR results indicated that mRNA expression levels of HP, LTF, and SERPING1 were also significantly elevated in the SZ group compared to the HC group (**Figure 5**).

Significantly higher expression levels of the SERPING1 gene were verified by WB in all clinical participant samples

This study included 150 gender- and age-matched SZ patients and 150 HCs to validate SERPING1 protein expression using WB (including three samples of the results shown in Figure 4a) . SERPING1 protein expression was significantly upregulated in the SZ group compared to the HC group (**Figures 6a and 6b**). The cohort of 150 SZ patients comprised 2 cases of simple type, 56 cases of undifferentiated type, and 92 cases of paranoid type. One-way ANOVA of SERPING1 protein expression among different SZ subtypes revealed no statistically significant differences (**Figure 6c**).

Significant reduction in the expression level of SERPING1 in neuronal cells in the CA1 region of rat hippocampus after lentivirus transfection

Transfection of target cells with pre-constructed RNAi-silencing lentivirus resulted in significant downregulation of SERPING1 mRNA expression in all three silencing targets (si-SERPING1#1, si-SERPING1#2, and si-SERPING1#3) compared to the negative control (si-NC) group. Among these, si-SERPING1#1 exhibited the highest silencing efficiency (**Figure 7**), thus it was selected for subsequent experiments.

Significant increase in the expression level of the nerve-related conductive proteins caMKII, CREB, and BDNF after lentiviral transfection of neuronal cells in the CA1 region of the rat hippocampus

Transfection of target cells with the si-SERPING1#1 silencing lentivirus significantly increased the expression of neuro-related signaling proteins, including caMKII (calcium/calmodulin-dependent protein kinase II), CREB (cAMP response element-binding protein), and BDNF (brain-derived neurotrophic factor), compared to the negative control (si-NC) group (**Figure 8**).

Significant decrease in apoptosis and increase in cell viability of neuronal cells in the CA1 region of rat hippocampus after lentiviral transfection

Transfection of target cells with the si-SERPING1#1 silencing lentivirus significantly reduced the apoptosis rate and increased cell viability compared to the NC negative control group (**Figures 9 and 10**, respectively). These results indicate that SERPING1 silencing promotes the proliferation of rat hippocampal neurons and reduces cell apoptosis.

DISCUSSION

The search for a reliable biomarker for early diagnosis of schizophrenia has been a topic of interest. However, until now, the identification of blood biomarkers in the study of major psychiatric disorders has been few and has failed to meet clinical needs[17]. Relevant studies have shown that peripheral blood mononuclear cells and brain tissues have similar expression patterns in some signal transduction and metabolic pathways[18]. Similarly, abnormal expression of synaptic genes highly associated with schizophrenia was also detected in peripheral blood mononuclear cells of patients with first-time episodes of schizophrenia[19]. E. Kozłowska *et al.*[20] found in their study on a case-control group of schizophrenia that peripheral blood mononuclear cells (PBMCs) are involved in the production of cytokines stimulated by spontaneous and plant hemagglutinin (PHA), indicating that immune system dysfunction may be the basis of the pathophysiology of schizophrenia. P. Petrikis *et al.*[21] conducted a systematic phosphorylation analysis of Akt, GSK3- β , and S6 in fresh peripheral blood mononuclear cells of first-episode psychiatric patients (FEP) and control subjects. The results showed that FEP patients had two different signaling endophenotypes, namely GSK3- β functional decline, which showed a mixed association with psychopathology and returned to normal after treatment, while mTORC1 functional decline represented a stable state. Their research provides new insights into the peripheral dysfunction of the Akt/GSK3- β /mTORC1 pathway. So, this study integrated transcriptomics and proteomics for a comprehensive analysis of PBMC samples from SZ patients. The

integration of these two omics data revealed that HP, LTF, and SERPING1 genes are upregulated in SZ patients. Moreover, the enrichment analysis suggests that neuroactive ligand-receptor related pathways may play an important role in the progression of SZ disease. This upregulation was validated by qRT-PCR and WB, which demonstrated significantly elevated mRNA and protein expression of these three genes in SZ patients. Large sample verification confirmed that the expression level of SERPING1 gene was significantly increased in SZ patients. In vitro silencing of the SERPING1 gene significantly increased the expression levels of neuronal pathway-related proteins CaMKII, CREB, and BDNF, reduced neuronal cell apoptosis, and enhanced cell viability. These findings suggest that the SERPING1 gene may influence the pathogenesis of SZ through the CaMKII-CREB-BDNF pathway.

The SERPING1 gene, located on chromosome 11 (11q12-q13.1), encodes the plasma protease C1 inhibitor (C1-Inh), a major inhibitor of the classical complement pathway and a known coagulation inhibitor. C1-Inh suppresses various inflammatory and coagulation pathways by inhibiting both the coagulation system and the complement system[22]. Research on SERPING1 has predominantly focused on hereditary angioedema, where C1-Inh deficiency, encoded by the SERPING1 gene, underlies the pathogenesis of types I and II hereditary angioedema[23]. Numerous SERPING1 variants have been identified, including single nucleotide polymorphisms, small insertions or deletions, and large insertions or duplications[24]. This study found upregulated expression of the SERPING1 gene in SZ but did not investigate the gene's structure, leaving the presence of pathogenic variants in SZ to be explored in future studies. Cooper JD *et al.* conducted a quantitative analysis of blood proteins from patients with first-episode SZ and found significant upregulation of hemoglobin and plasma protease C1 inhibitor[25], consistent with our proteomics findings. A transcriptomics analysis of post-mortem brain tissue from SZ patients identified upregulation of several genes involved in immune response and inflammation, including SERPING1, suggesting that elevated complement cascade components are significant in SZ pathology[26]. Allswede DM *et al.* analyzed PBMC samples from adult

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SZ twins in Sweden ($n = 129$), finding that peripheral mRNA expression levels of complement genes C5 and SERPING1 uniquely contributed to differences in frontal cortical thickness among participants[27].

The haptoglobin (HP) gene, located on chromosome 16 (16q22), belongs to a multigene family with two alleles, HP1 and HP2, encoding HP1 and HP2 proteins, respectively[28]. Research by WAN C *et al.*[29] found altered HP protein expression and different genotype distributions in SZ patients in the Chinese Han population, indicating an association between HP and SZ. Lee *et al.* reported upregulated HP gene expression in patients with first-episode psychiatric disorders. The lactotransferrin (LTF) gene, located on chromosome 3 (3p21) and also known as lactoferrin (LF), is part of the transferrin family. LTF is a multifunctional protein involved in antibacterial, antiviral, and anti-inflammatory processes, regulating lipid metabolism, preventing oxidative stress, playing a role in cell growth and immunity, and participating in various enzymatic reactions[30]. Simeonova D *et al.*[31] reported significantly elevated serum LTF levels in SZ patients, unaffected by antipsychotic therapy. This study found significant upregulation of the LTF gene in both transcriptomics and proteomics analyses, highlighting its relevance to SZ. However, research on the LTF gene in SZ is limited, necessitating further exploration to understand its role in SZ and providing new directions for future research.

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The brain-derived neurotrophic factor (BDNF) gene, located on chromosome 11p13 and spanning 70kb with 11 exons[32], has been found to have reduced levels in both first-episode and chronic SZ patients. This reduction suggests that BDNF may serve as a biomarker for cognitive impairment in SZ[33]. Studies on the Han Chinese population have shown that BDNF gene polymorphisms are associated with negative symptoms of SZ[34]. Neurotrophic factors like BDNF play a crucial role in neurodevelopment and synaptic plasticity[35, 36]. The cAMP response element-binding protein (CREB) is a major regulator of neurotrophic factor response; phosphorylated CREB binds to specific sequences in the BDNF promoter to regulate its transcription[37]. Guo *et al.*[38] reported that increased BDNF mRNA expression parallels the increase in phosphorylated CREB

expression. Calcium/calmodulin-dependent protein kinase II (CaMKII) in the hippocampus is essential for learning and memory consolidation. Recent studies suggest that reduced CaMKII activity may be a fundamental cause of widespread brain dysfunction, including various neuropsychiatric disorders such as addiction, SZ, depression, epilepsy, and multiple neurodevelopmental disorders. This is likely due to impaired glutamate signal transduction and neural plasticity[39]. Research on CaMKII α heterozygous knockout mice has shown characteristic EEG and behavioral changes seen in subtypes of SZ and intellectual disability[40]. Studies on rodents have also demonstrated the potential of enhancing CaMKII activity to treat cognitive impairments in SZ[41]. Silencing the SERPING1 gene significantly increased the expression of neuronal pathway proteins CaMKII, CREB, and BDNF. This suggests that SERPING1 may influence SZ through the CaMKII-CREB-BDNF signaling pathway, making it a potential novel target for SZ diagnosis and treatment. Additionally, Jung G *et al.* demonstrated that the atypical antipsychotic olanzapine upregulates BDNF gene transcription by enhancing CREB transcription *via* the PKA, PI3K, PKC, and CaMKII signaling pathways, thereby exerting neuroprotective effects[42]. Cognitive impairment in SZ patients is closely linked to synaptic damage. A study inducing synaptic damage in rats suggested that neuronal injury may be associated with the CaM/CaMKII/CREB/BDNF signaling pathway, which involves postsynaptic membrane ion channel proteins and molecules related to synaptic plasticity[43]. Theobromine (TB) treatment in rats improved working memory by upregulating the CaMKII/CREB/BDNF pathway in the medial prefrontal cortex[44]. Although current research on the regulation of the CaMKII-CREB-BDNF pathway predominantly focuses on depression, studies specific to this pathway in SZ are relatively scarce.

This study utilized WB to detect SERPING1 protein expression in PBMCs from 150 SZ patients and 150 HCs and compared the results among different disease subtypes. Results indicated that SERPING1 protein expression was significantly higher in SZ patients compared to the HC group. However, no significant difference was found in SERPING1 protein expression among the different subtypes, potentially due to sample

size limitations. SZ is a heterogeneous disorder influenced by multiple genes and factors and does not follow Mendelian inheritance patterns. The inconsistency between genotype and disease phenotype could explain the lack of significant correlation between SERPING1 protein levels and clinical subtypes. In this study, an RNAi-silenced lentivirus targeting the SERPING1 gene was constructed and transfected into rat neuronal cells. Silencing the SERPING1 gene significantly promoted neuronal cell proliferation and inhibited apoptosis. Additionally, silencing led to the upregulation of channel proteins CaMKII, CREB, and BDNF, validating the neuroprotective effect of the CaMKII-CREB-BDNF signaling pathway. This study is among the few to investigate the role of the SERPING1 gene in neuronal cell proliferation and apoptosis. The limitations of this study include the fact that all enrolled cases were recurrent SZ patients who had long-term antipsychotic treatment, potentially introducing confounding factors in the peripheral blood omics analysis. Furthermore, the relatively small sample size may result in sample selection bias, limiting the generalizability of the findings to the broader SZ population.

CONCLUSION

In summary, this study integrated transcriptomics and proteomics to identify significant upregulation of the genes HP, LTF, and SERPING1 in the PBMCs of SZ patients. Validation with clinical samples confirmed a significant increase in SERPING1 gene expression levels. Silencing the SERPING1 gene resulted in a notable upregulation of the neural pathway-associated channel proteins CaMKII, CREB, and BDNF. Cell experiments demonstrated that the SERPING1 gene has anti-proliferative and pro-apoptotic effects on neuronal cells. Overall, the SERPING1 gene may inhibit neuronal cell proliferation through the CaMKII-CREB-BDNF pathway, thereby influencing the development and progression of SZ. This finding could provide new insights for future research and potential therapeutic targets for the disease.

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