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Transcriptomic and proteomic analyses of SH-SY5Y neuroblastoma cells treated with amisulpride

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Original Article

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Abstract

Objective: Amisulpride, a substituted benzamide derivative, has a unique pharmacological profile characterised by a high affinity for dopaminergic D_2/D_3 receptors, as well as an affinity for 5-HT_7 receptors. Its effectiveness and safety surpass those of traditional antipsychotic drugs and multi-receptor antipsychotic medications in improving global symptoms, including both positive and negative symptoms. This makes it a compelling subject for study. However, the molecular mechanisms that contribute to its clinical efficacy in treating schizophrenia remain largely unexplored. *Methods:* We assessed cell viability following amisulpride treatment using the MTT and a real-time cell viability assay. Subsequently, we conducted RNA-seq and LC-MS/MS analyses to identify differentially expressed genes and proteins in SH-SY5Y neuroblastoma cells treated with amisulpride. *Results:* In the present study, we used RNA-seq analysis to identify downregulated expression of a transcriptional factor, *FOSB*, in amisulpride-treated SH-SY5Y neuroblastoma cells, while using LC-MS/MS analysis to identify multiple differentially expressed proteins in these cells. Among these differentially expressed proteins, we confirmed four proteins (ACTG1, ANP32E, CLTC, IPO8) that are differentially expressed under the administration of amisulpride. *Conclusion:* Our data reveal novel insights into the role of amisulpride in modulating the differential expression of genes and proteins. These findings, which involve genes/proteins related to AP-1 transcription factor family gene regulation, cytoskeleton, histone binding activity, the intracellular trafficking of receptors and endocytosis of a variety of macromolecules, and nuclear localisation signal, are particularly significant as they shed light on the molecular underpinnings of the clinical efficacy of amisulpride and the pathogenesis of schizophrenia.

Significant outcomes

- Identifying differentially expressed genes and proteins enhances our understanding of the molecular mechanisms behind amisulpride's clinical effects.
- It offers potentially new pathogenic mechanisms and treatment targets for schizophrenia.

Limitations

- The sample size is small and was conducted using only the SH-SY5Y cell line.
- Other genes and proteins need to be verified to gain more insight into the molecular mechanism of amisulpride.

Introduction

The effectiveness of traditional antipsychotic medications is linked to their ability to block dopamine D_2 receptors (Willner, 1997). Haloperidol is a traditional antipsychotic drug that acts as a complete dopamine D_2 receptor antagonist. It is effective in alleviating schizophrenia symptoms but may cause side effects, including extrapyramidal symptoms and elevated prolactin levels (Beasley *et al.*, 1999). A broader spectrum of therapeutic efficacy of atypical antipsychotic drugs such as risperidone, olanzapine, and clozapine has been introduced to the market for treating schizophrenia (Newcomer, 2005). An atypical antipsychotic drug known as amisulpride is a substituted benzamide derivative that possesses a unique pharmacological profile, characterised by a high affinity for dopaminergic D_2/D_3 and an affinity for 5-HT_7 receptors (Perrault *et al.*, 1997; Moller, 2003). Amisulpride is more effective and safer than traditional and multi-receptor antipsychotics in improving global

symptoms, including both positive and negative symptoms of schizophrenia (Komossa *et al.*, 2010). Currently, there is still a limited understanding of the molecular mechanisms that contribute to the clinical effectiveness of amisulpride in treating schizophrenia.

Proteogenomics is a powerful strategy for gene and protein expression profiles in neuroscience and neurology (Nesvizhskii, 2014). For example, transcriptomic and proteomic analyses of antipsychotic drugs have identified genes associated with schizophrenia. (Bortolasci *et al.*, 2020; Truong *et al.*, 2022). A recent genome-wide mRNA expression study showed that eight antipsychotic drugs downregulate the expression of genes related to the focal adhesions pathway (Panizzutti *et al.*, 2025). This suggests that adhesion pathways may play a role in the pathophysiology of bipolar disorder and schizophrenia.

However, many detailed molecular mechanisms of amisulpride's action remain unknown. In this study, we performed RNA sequencing (RNA-seq) and LC-MS/MS analysis to identify the differentially expressed genes (DEGs) and proteins in SH-SY5Y neuroblastoma cells treated with amisulpride. A more precise understanding of the molecular effects of amisulpride on SH-SY5Y cells could offer insights into the molecular mechanisms underlying psychiatric disorders, potentially revealing novel treatment targets.

Materials and methods

Cell culture and amisulpride treatment

The human SH-SY5Y neuroblastoma cell line (Sigma catalogue no. 94030304) was cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. The SH-SY5Y cells were cultured in a humidified environment with 5% CO_2 at 37°C, and the medium was refreshed every two to three days. Amisulpride was sourced from Sigma–Aldrich (A2729). A stock solution was made in dimethyl sulfoxide (DMSO) and subsequently diluted in the medium to achieve the desired final concentration. The concentrations of amisulpride used in this study were selected based on previous research examining its effects on the SH-SY5Y cells (Park *et al.*, 2011). We administered doses of 0.4, 4, 20, and 40 µg/ml, which are within a pharmacologically relevant range known to modulate the activity of D_2/D_3 and 5-HT_7 receptors without causing cytotoxicity. A 24–hour treatment period was chosen to allow sufficient time for amisulpride-induced changes in the transcriptome and proteome while minimising downstream secondary stress responses or cell death. To ensure statistical robustness and reproducibility of the transcriptomic and proteomic data, we performed three independent biological replicates per condition, with each set derived from separate cell culture passages.

MTT assay

The SH-SY5Y cells were cultured in 96-well plates at a density of 1.5×10^4 cells per well and were incubated for 24 h. After this initial incubation, the cells were treated with amisulpride at various concentrations for 24 h in serum-free DMEM. Following treatment, the cells were washed twice with phosphate-buffered saline (PBS) and then cultured in DMEM supplemented with 10% FBS for two days. Subsequently, the cells were incubated with 0.5 mg/ml of the MTT (Sigma Chemical Co., St Louis, MO, USA) in DMEM for 4 h. Viable cells converted MTT to formazan, which appears blue-purple when dissolved in DMSO. The intensity of the colour, measured as absorbance, is directly proportional to the number of live cells. Absorbance at 545 nm was recorded using a microplate reader (Varioskan Flash, Thermo Fisher Scientific, Vantaa, Finland). The percentage of cell survival was calculated by dividing the absorbance of the amisulpride-treated samples by the absorbance of the corresponding DMSO-treated controls.

Real-time cell viability assay

Real-time cell viability was conducted using the RealTime-Glo™ MT Cell Viability Assay (Cat.# G9711, Promega). The SH-SY5Y cells (1×10^4 per well) were plated in a 96-well plate and cultured in a 5% CO_2 atmosphere at 37°C overnight to ensure thorough incubation conditions. The medium was then replaced with a growth medium containing, various dosages of amisulpride, the MT Cell Viability Substrate (Promega) and NanoLuc Enzyme (Promega), and the cells were cultured for 72 h. Luciferase activity was measured using a microplate reader (VANTAstar™, BMG LABTECH, Germany).

Total RNA preparation, RNA-seq, and RT-qPCR

Total RNA preparation, RNA-seq, RT-qPCR, and DEG identification were performed following the methods described in a previous study (Wang *et al.*, 2022). The target gene, *FOSB* (Hs00171851_m1, FAM™ dye-labelled TaqMan™ MGB probe), and two endogenous genes, *GAPDH* (Hs02786624_g1, VIC™ dye-labelled TaqMan™ MGB probe) and *18S* (Hs99999901_s1, VIC™ dye-labelled TaqMan™ MGB probe), were analysed using TaqMan™ gene expression assays according to the manufacturer's protocol (ThermoFisher Scientific Inc.). The expression levels of *ACTG1*, *ANP32E*, *CLTC*, and *IPO8* were assayed using SYBR Green detection (ThermoFisher Scientific Inc.), and the primer sequences are listed in the Supplementary Table S1. All tests were performed in six replicates. Statistically significant differences between the treated and control groups were determined using a p–value threshold of <0.05.

Protein sample preparation and LC-MS/MS analysis

To prepare protein samples, cells were washed twice with cold PBS and then resuspended in a lysis buffer that contain 20 mM HEPES (pH 7.6), 7.5-mM NaCl, 2.5 mM $MgCl_2$, 0.1 mM EDTA, 0.1% TritonX-100, 50 mM NaF, 0.1 mM Na_3VO_4, and a protease inhibitor cocktail (one mini-tablet/10 ml; Roche Diagnostics GmbH). The homogenates were centrifuged at 13,000 r.p.m. for 30 minutes at 4°C, and the resulting supernatants were stored at -80°C until needed.

In the process of solution digestion, LC-MS/MS and protein identification were conducted by BIOTOOLS CO., LTD in Taiwan. The procedure began with diluting the protein solutions in 50 mM ammonium bicarbonate (ABC, Sigma). The samples were then reduced with 10 mM dithiothreitol (DTT, Merck) at 56°C for 45 minutes. Following this, cysteine residues were blocked with 50 mM iodoacetamide (IAM, Sigma) at 25°C for 30 minutes. Next, the samples were digested with sequencing-grade modified porcine trypsin (Promega) at 37°C for 16 h. After digestion, the peptides were desalted, dried by vacuum centrifugation, and stored at -80°C until further use.

In the meticulous LC-MS/MS analysis process, the digested peptides were diluted in HPLC buffer A (0.1% formic acid) and

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loaded onto a reverse-phase column (Zorbax 300 SB-C18, 0.3 × 5 mm; Agilent Technologies). The desalted peptides were separated using a column (Waters BEH 1.7 µm, 100 µm I.D. × 10 cm with a 15 µm tip) and a multi-step gradient of HPLC buffer B (99.9% acetonitrile/0.1% formic acid) over a period of 70 minutes, at a flow rate of 0.3 µl/min. The liquid chromatography apparatus was coupled with a 2D linear ion trap mass spectrometer (Orbitrap Elite ETD; Thermo Fisher), which was operated using Xcalibur 2.2 software (Thermo Fisher). The full-scan MS was performed in the Orbitrap over a range of 400 to 2,000 Da, with a a resolution of 120,000 at m/z 400. Internal calibration was achieved using the ion signal of protonated dodecamethylcyclohexasiloxane ion at m/z 536.165365 as the lock mass. The analysis included 20 data-dependent MS/MS scan events, each followed by one MS scan for the 20 most abundant precursor ions in the preview MS scan. The m/z values selected for MS/MS were dynamically excluded for 40 s with a relative mass window of 15 ppm. The electrospray voltage was set to 2.0 kV, and the capillary temperature was maintained at 200°C. Automatic gain control for MS and MS/MS was set to 1,000 ms (for full scan) and 200 ms (for MS/MS), or 3×10^6 ions (full scan) and 3,000 ions (MS/MS) for maximum accumulated time or ions, respectively.

The protein identification was conducted using Proteome Discoverer software (version 2.3, Thermo Fisher Scientific). The MS/MS spectra were searched against the UniProt database utilising the Mascot search engine (Matrix Science, London, UK; version 2.5). For peptide identification, a mass tolerance of 10 ppm was allowed for intact peptide masses and 0.5 Da for CID fragment ions, with allowance for two missed cleavages resulting from the trypsin digestion. The variable modifications included oxidised methionine and acetylation at the protein N-terminal, while carbamidomethylation of cysteine was used as a static modification. Peptide-spectrum matches (PSM) were filtered based on high confidence, and the Mascot search engine ranked the top identification for each peptide, ensuring that the overall false discovery rate below 0.01. Proteins identified by only a single peptide hit were excluded from the final results.

Bioinformatic analysis

For Gene Ontology (GO) enrichment and pathway analysis, differentially expressed proteins between amisulpride-treated and non-treated samples ($P < 0.05$) were subjected to the Database for Annotation, Visualisation, and Integrated Discovery (DAVID, https://davidbioinformatics.nih.gov/) using the official gene symbol method.

Immunoblotting

Immunoblotting was conducted following standard protocols, utilising the primary antibodies listed below. Rabbit anti-ACTG1 (11227–1-AP; Proteintech, Rosemont, IL, USA), rabbit anti-ANP32E (A17220; ABclonal, Woburn, MA, USA), rabbit anti-CLTC (A12423; ABclonal), rabbit anti-CRMP1 (A2705; ABclonal), rabbit anti-HSD17B10 (A5448; ABclonal), rabbit anti-IPO8 (A14679; ABclonal), rabbit anti-NPEPPS (A08129–2; Boster Biological Technology, Pleasanton, CA, USA), rabbit anti-OTUB1 (A11656; ABclonal), rabbit anti-PRMT1 (A1055; ABclonal), rabbit anti-RAC1 (A7720; ABclonal), rabbit anti-SPTAN1 (A0160; ABclonal), rabbit anti-SEPTIN11 (A12189; ABclonal), rabbit anti-TIA1 (A12523; ABclonal), and mouse anti-GAPDH (G8795; Sigma–Aldrich, Saint Louis, MO, USA). Horseradish peroxidase-conjugated donkey anti-rabbit IgG (NA934V, GE Healthcare Life Sciences, UK) and human anti-mouse IgG (5220–0341; KPL) were used as secondary antibodies. Chemiluminescence was visualised using an enhanced chemiluminescence detection system (GTX400006; GeneTex).

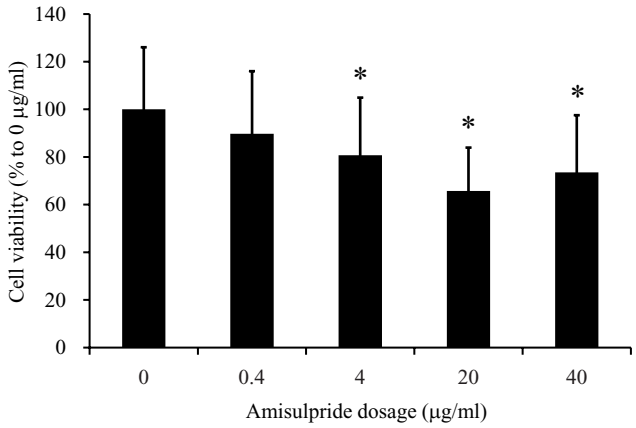

Figure 1. Amisulpride exhibits concentration-dependent effects on the viability of SH-SY5Y cells. The MTT assay was conducted to estimate cell numbers after a 24–hour treatment with varying concentrations of amisulpride. The results were calculated as the optical density at 545 nm (OD545) of amisulpride-treated cultures compared to non-treated control cultures and expressed as means ± standard deviation. A statistically significant difference between amisulpride-treated and non-treated cultures was identified using ANOVA, with post hoc tests performed using by LSD method (*p < 0.01, $n = 80$).

Results

Effects of amisulpride on SH-SY5Y cell viability

The effects of a 24–hour amisulpride treatment (0.4, 4, 20, and 40 µg/ml) on SH-SY5Y cell viability were examined using the MTT assay (Fig. 1). The survival rates compared to 0 µg/ml controls were 81% ($p = 3E-06$) after exposure to 4 µg/ml amisulpride, 66% ($p = 1.4E-17$) after exposure to 20 µg/ml, and 74% ($p = 4.1E-09$) after exposure to 40 µg/ml amisulpride.

We measured cell viability over the 72 h of amisulpride treatment using the RealTime-Glo™ MT Cell Viability Assay. Our results confirmed that amisulpride at concentrations of 4, 20, and 40 µg/ml) inhibited cell survival compared to the 0 µg/ml control group (Fig. 2).

Identification of differentially expressed genes by transcriptome sequencing

Nine samples from three biological replicates (with doses of 0, 20, and 40 ug/ml of amisulpride) were used for transcriptome sequencing. The number of reads per sample ranged from 38,707,688 to 51,321,892 across the nine sequenced RNA samples (Supplementary Table S2). Differential gene expression analysis revealed that the *FOSB* gene was significantly downregulated in the group treated with 20 µg/ml amisulpride group, with a fold change greater than 2 and an adjusted *p*-value of 7.46E-11 (Supplementary Table S3 and S4). The mRNA expression level of *FOSB* was confirmed in biologically replicated SH-SY5Y cells using a real-time quantitative PCR (RT-qPCR) assay (Fig. 3).

Protein ID, bioinformatic analysis, immunoblot verification, and RT-qPCR analysis

We utilised a label-free LC-MS/MS shotgun proteomics strategy to identify proteins that were differentially expressed in the SH-SY5Y

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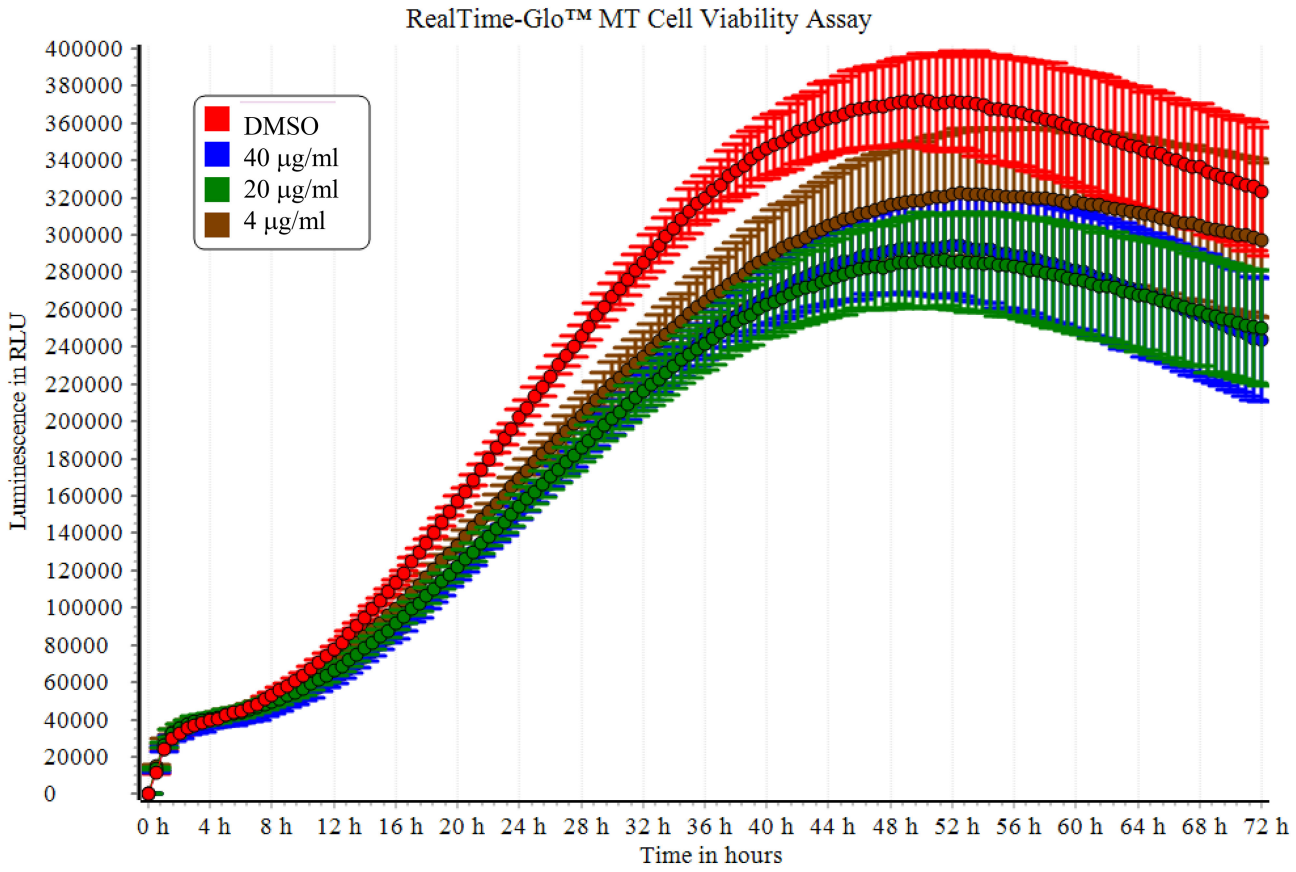

Figure 2. Analysis of amisulpride-treated SH-SY5Y cells with RealTime-Glo™ MT Cell Viability. Real-time cell viability (luminescent signals) was monitored every 30 minutes for 72 h on a VANTAstar™ microplate reader with a gas control module (37°C and 5% CO_2) ($n = 6$).

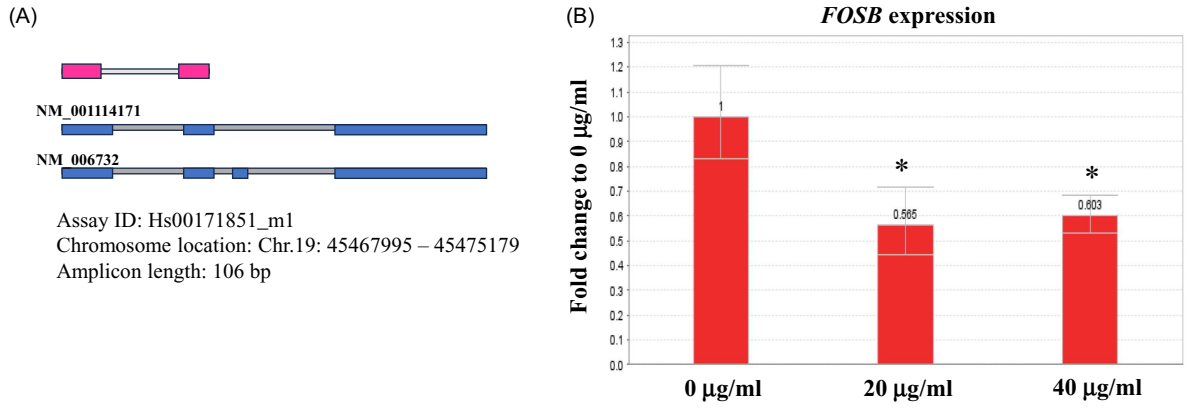

Figure 3. The mRNA expression level of *FOSB* in amisulpride-treated SH-SY5Y cells. (A) a schematic representation of the *FOSB* gene genomic map, including primers and probes (pink rectangle) used for the TaqMan assay (Hs00171851_m1 from Thermo Fisher Scientific). (B) RT-qPCR assay results showing the expression of *FOSB* in SH-SY5Y cells treated with amisulpride. GAPD*H* was used as an endogenous gene for normalisation. Data are expressed as fold changes relative to 0 µg/ml of amisulpride ± standard deviation (* $p < 0.05$; $n = 6$).

cells treated with amisulpride. We analysed nine samples from three biological replicates at three different doses of amisulpride: 0, 20, and 40 µg/ml. Normalised peptide-spectrum matches (PSMs) were calculated using the following formula: (PSM in sample A/ total PSM in sample A) × average PSM across all nine samples. Supplementary Table S5 lists the protein accession numbers, descriptions, and normalised PSMs of the differentially expressed proteins between amisulpride-treated and non-treated samples. To evaluate the differences in normalised PSMs between the two groups, we conducted Student's t-tests with a significance threshold of $P < 0.05$.

GO enrichment and pathway analysis revealed that the differentially expressed proteins between amisulpride-treated and non-treated samples were significantly associated with several GO terms and three pathways after applying the Bonferroni adjustment (Table 1, P<0.05). Thirteen proteins that exhibited differential expression between the groups were selected for further verification using immunoblot analysis on independent biological

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Table 1. Summary of GO enrichment and pathway analyses in differentially expressed proteins between amisulpride-treated and non-treated samples

Category	Term	Protein count	Protein IDs	Bonferroni P-value
BP	GO:0002181~cytoplasmic translation	7	*RPS14, RPL5, RPL32, RPL35A, RPL35, RPS10, RPL6*	3.22E−04
BP	GO:0006412~translation	8	*RPS14, RPL5, RPL32, NARS1, RPL35A, RPL35, RPS10, RPL6*	0.0054
BP	GO:0006418~tRNA aminoacylation for protein translation	4	*GARS1, NARS1, LARS1, IARS1*	0.0348
CC	GO:0005737~cytoplasm	50	*RPL5, RPL32, GDI2, HMGB2, NUCKS1, YBX1, ACTB, RPL6, ACTG1, EEF1B2, NPEPPS, RPS14, NARS1, SCP2, RPL35, RAC1, SPTAN1, RPS10, ANXA2, PRMT1, ACTN1, CTPS1, ACTN4, TUBA4B, DNM2, ACTA1, LARS1, PSME3, ARL3, PHYHIPL, CRMP1, HSD17B10, MYG1, HNRNPDL, PRKAR2B, CBS, DDT, IARS1, TIA1, ALYREF, HUWE1, RPL35A, NUDT21, GARS1, GLUD1, EIF5, COPS2, HNRNPA2B1, ANP32E, ADSS2*	6.83E-12
CC	GO:0070062~extracellular exosome	32	*RPL5, ARL3, GDI2, CLTC, YBX1, ACTB, ACTG1, RPS14, NPEPPS, NARS1, PRKAR2B, DDT, PSMD2, IARS1, RAC1, ATP6V1E1, SPTAN1, TXNDC5, ANXA2, ALYREF, ACTN1, HUWE1, RPL35A, ACTN4, CYRIB, TUFM, DNM2, GARS1, ACTA1, HNRNPA2B1, ADSS2, OTUB1*	2.83E-11
CC	GO:0005829~cytosol	46	*RPL5, RPL32, USO1, GDI2, CLTC, CRMP1, YBX1, RPL6, ACTB, ACTG1, EEF1B2, RPS14, NPEPPS, IPO8, HNRNPDL, NARS1, SCP2, RBBP4, PRKAR2B, CBS, PSMD2, RPL35, IARS1, RAC1, ATP6V1E1, ENOPH1, SPTAN1, RPS10, TIA1, USP5, ANXA2, PRMT1, ALYREF, ACTN1, HUWE1, RPL35A, CTPS1, DNM2, GARS1, ACTA1, EIF5, LARS1, PSME3, COPS2, ADSS2, OTUB1*	1.52E-09
CC	GO:1990904~ribonucleoprotein complex	12	*RPS14, RPL5, TIA1, RPL32, HNRNPDL, HNRNPA2B1, RPL35A, ACTN4, YBX1, RPS10, RPL6, ACTB*	4.36E-07
CC	GO:0005925~focal adhesion	12	*RPS14, RPL5, ACTN1, GDI2, CLTC, ACTN4, RAC1, RPS10, RPL6, ACTB, ACTG1, DNM2*	2.50E-05
CC	GO:0022626~cytosolic ribosome	7	*RPS14, RPL5, RPL32, RPL35A, RPL35, RPS10, RPL6*	1.48E-04
CC	GO:0022625~cytosolic large ribosomal subunit	5	*RPL5, RPL32, RPL35A, RPL35, RPL6*	0.0074
CC	GO:0045202~synapse	10	*EIF5, RPL32, GDI2, RPL35A, RAC1, YBX1, ACTB, TUFM, ACTG1, DNM2*	0.0098
MF	GO:0003723~RNA binding	26	*RPL5, RPL32, USO1, GDI2, CLTC, HMGB2, NUCKS1, YBX1, HSD17B10, RPL6, RPS14, HNRNPDL, RPL35, EIF3CL, RPS10, TIA1, ANXA2, PRMT1, ALYREF, HUWE1, RPL35A, ACTN4, TUFM, NUDT21, EIF5, HNRNPA2B1*	1.56E-09
MF	GO:0005515~protein binding	62	*RPL5, HIBADH, CLTC, GDI2, HMGB2, NUCKS1, YBX1, ACTB, SEPTIN11, RPL6, ACTG1, EEF1B2, RPS14, MYDGF, IPO8, NARS1, SCP2, PSMD2, EIF3CL, RAC1, ATP6V1E1, SPTAN1, RPS10, ENOPH1, USP5, ANXA2, PRMT1, ACTN1, CTPS1, ACTN4, TUFM, DNM2, ACTA1, LARS1, PSME3, HAT1, OTUB1, ARL3, USO1, PHYHIPL, CRMP1, HSD17B10, MYG1, HNRNPDL, RBBP4, PRKAR2B, CBS, IARS1, TXNDC5, TIA1, ALYREF, HUWE1, RPL35A, CYRIB, NUDT21, GARS1, GLUD1, EIF5, COPS2, HNRNPA2B1, ANP32E, ADSS2*	0.0053
MF	GO:0003735~structural constituent of ribosome	7	*RPS14, RPL5, RPL32, RPL35A, RPL35, RPS10, RPL6*	0.0120
MF	GO:0005525~GTP binding	9	*GLUD1, EIF5, ARL3, RAC1, ADSS2, TUBA4B, SEPTIN11, TUFM, DNM2*	0.0188
KEGG	hsa05100: Bacterial invasion of epithelial cells	6	*CLTC, RAC1, SEPTIN11, ACTB, ACTG1, DNM2*	0.0051
KEGG	hsa03010: Ribosome	7	*RPS14, RPL5, RPL32, RPL35A, RPL35, RPS10, RPL6*	0.0249
BIOCARTA	h_uCalpainPathway: uCalpain and friends in Cell spread	4	*ACTA1, ACTN1, RAC1, SPTAN1*	0.0270

BP: biological process; CC: cellular component; MF: molecular function; KEGG: Kyoto Encyclopaedia of Genes and Genomes (https://www.genome.jp/kegg/).

replicates of the SH-SY5Y cells (Fig. 4A). We determined the fold differences in the expression of the selected proteins (Fig. 4B), and confirmed the differential expression of four proteins: ACTG1, ANP32E, CLTC, and IPO8. We compared the mRNA expression levels of four genes (*ACTG1*, *ANP32E*, *CLTC*, and *IPO8*) in biologically replicated SH-SY5Y cells treated with amisulpride (0, 20, and 40 μg/ml) using RT-qPCR assay. We did not find significant differences in expression among these four genes across the three groups (Supplementary Figure S1).

Discussion

Antipsychotic medications are the preferred treatment of choice for patients experiencing psychotic symptoms, including delusions, hallucinations, bizarre behaviour, agitation, and aggression. Research indicates that changes in neural plasticity, driven by differential gene expression in the brain, may support the clinical effectiveness of antipsychotic medications (Hyman & Nestler, 1996). In this study, we conducted a transcriptomic analysis to discover a

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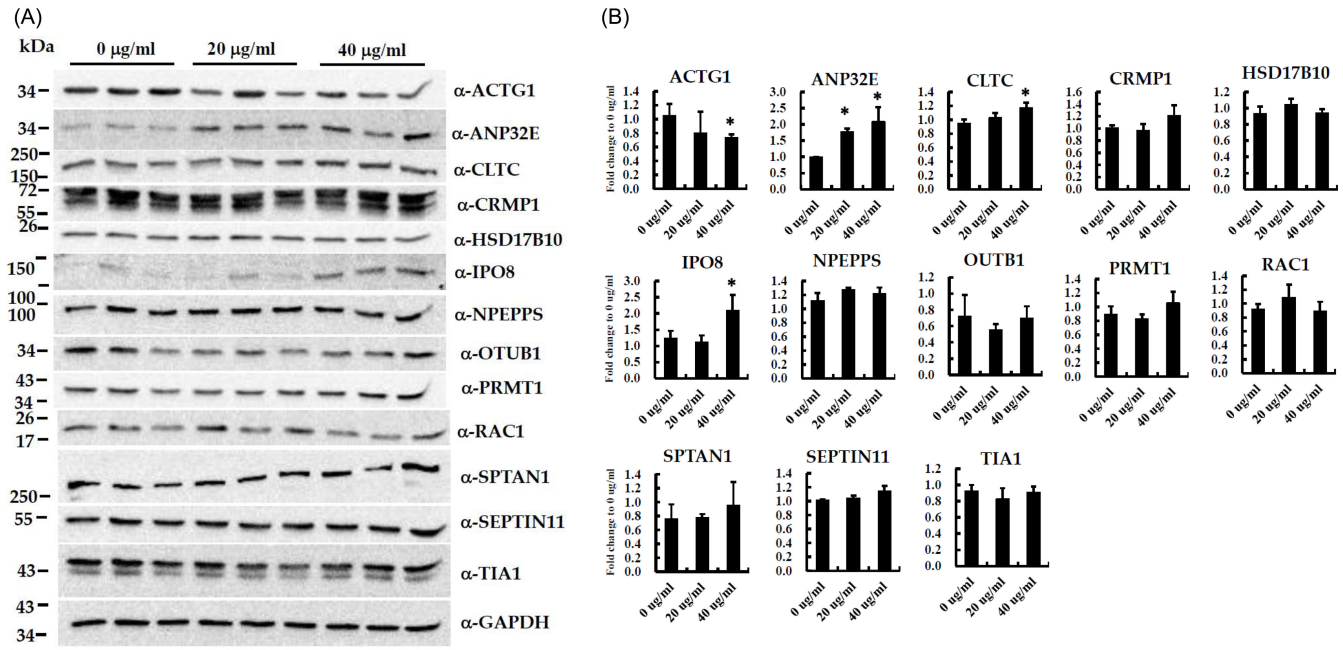

Figure 4. Immunoblotting analysis to validate the differential expression for 13 proteins in amisulpride-treated biological replicated SH-SY5Y cells. (A) immunoblotting and (B) quantification of protein expression showing the fold differences of four proteins (ACTG1, ANP32E, CLTC, IPO8) between amisulpride-treated groups and control. GAPDH was a loading control. Data are expressed as fold change to 0 ug/ml of amisulpride ± standard deviation (*$p < 0.01$, $n = 3$).

downregulated expression of a transcriptional factor, the *FOSB* gene, in SH-SY5Y neuroblastoma cells treated with amisulpride. We also performed proteomic analysis to identify several proteins differentially expressed in the amisulpride-treated cells, which involved pathways related to synapse, extracellular exosome, structural constituent of ribosome, RNA and protein binding, and bacterial invasion of epithelial cells. We confirmed four specific proteins (ACTG1, ANP32E, CLTC, and IPO8) among the differentially expressed proteins, which showed significant changes in expression due to amisulpride administration, using an immunoblotting assay.

The activator protein 1 (AP-1) transcription factor family is a crucial class of transcriptional regulators that control various aspects of cell physiology in response to environmental changes, including stress, cytokines, infections, oncogenic stimuli, and growth factors (Durchdewald *et al.*, 2009). Upon regulation, the AP-1 controls the expression of target genes associated with cell proliferation, differentiation, transformation, apoptotic cell death, and migration (Hisanaga *et al.*, 1990; Durchdewald *et al.*, 2009). The AP-1 family includes essential region leucine zipper (bZIP) domain proteins such as JUN, FOS, and FOSB. These proteins must dimerise to form the transcription factor complex AP-1 before binding to their DNA target sites (Wagner, 2001). Research suggests that the transcriptional activation of the AP-1 complex plays a vital role in the central nervous system, brain development, and psychiatry disorders (Pennypacker, 1995). For example, multiple studies have reported changes in fos and jun family genes as markers of transcriptional activity alterations caused by antipsychotic drug treatment (Kontkanen *et al.*, 2002; Kiss & Osacka, 2020). A study found that *FosB* mutant mice lost the induction of Fos-related proteins by chronic cocaine exposures and exhibited abnormal locomotor and conditioned place preference responses (Hiroi *et al.*, 1997). In this study, we observed a reduction in the gene expression of the *FOSB* gene in the SH-SY5Y cells treated with amisulpride. This suggests that the *FOSB* gene may play a role in the molecular mechanism of antipsychotic drugs and the pathophysiology of psychiatric disorders. Notably, two studies have reported an association between the *FOSB* gene and the pathogenesis of schizophrenia, although their findings are inconsistent. A genome-wide gene expression study has found that the *FOSB* gene was upregulated in fibroblast samples from individuals with schizophrenia (Huang *et al.*, 2019). In contrast, a transcriptomic study revealed that the *FOSB* gene is downregulated in the brain tissue of patients with schizophrenia (Chen *et al.*, 2023). The discrepancy might be attributed to differences in antipsychotic treatment and the types of tissue collected. Taken together, we propose that amisulpride's effects on the regulation of genes within the AP-1 transcription factor family are linked to mental illnesses that feature psychotic symptoms. Moreover, the *FOSB* gene was acceptable as a candidate gene for schizophrenia and may play a role in its pathogenesis.

Evidence indicates that abnormal synaptogenesis and synaptic dysfunction are crucial to the pathophysiology of schizophrenia, highlighting potential therapeutic targets for synaptic circuit modulation in psychiatric disorders (Howes & Onwordi, 2023; Wolf & Abi-Dargham, 2023). In this study, we found amisulpride treatment can increase the protein level of a synaptic vesicle-related protein, clathrin heavy chain (CLTC), in the SH-SY5Y cells. CLTC encodes clathrin heavy chain, a crucial component of clathrin-coated vesicles that mediate intracellular trafficking, especially endocytosis and membrane recycling (Narayana *et al.*, 2019; Itagaki & Kamei, 2025). Recent studies have shown that CLTC is also involved in synaptic transmission and the cycling of synaptic vesicles (Pannone *et al.*, 2023; Yang *et al.*, 2025a). Furthermore, several studies indicate that *CLTC* mutations are associated with childhood-onset schizophrenia, neurodevelopmental disorders, epileptic encephalopathy, and Parkinsonism (DeMari *et al.*, 2016; Manti *et al.*, 2019, Nabais Sá et al., 2020, Usnich *et al.*, 2024). Overall, dysfunction of CLTC may contribute to a wide range of neuropsychiatric risks. Therefore, CLTC could be a potential therapeutic target for treatment of psychiatric disorders.

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Mounting evidence underscores the pivotal role of actin remodelling in synaptogenesis, synaptic plasticity, and the intricate development of neurites in burgeoning neurons (Matus, 2000; Hotulainen & Hoogenraad, 2010). For instance, the dynamic nature of actin filaments is instrumental in forming dendritic spines during development, and they contribute significantly to the structural plasticity of mature synapses (Matus, 2000). A growing body of research has illuminated the regulatory mechanisms that finely tune actin dynamics within dendritic spines (Mattila & Lappalainen, 2008; Hotulainen & Hoogenraad, 2010). Notably, Kimoto and colleagues discovered that levels of transcripts associated with actin and mitochondrial oxidative phosphorylation are profoundly altered in individuals with schizophrenia (Kimoto *et al*., 2022). Furthermore, a study reports alterations in the dendritic spine across multiple brain regions in schizophrenia (Glausier & Lewis, 2013). The compelling evidence suggests that the abnormal morphology of dendritic spines observed in schizophrenia may indeed be linked to disruptions in the delicate regulation of actin cytoskeletal dynamics. In this study, we found that amisulpride treatment can decrease the protein level of ACTG1 in the SH-SY5Y cells. The *ACTG1* gene encodes cytoplasmic gamma actin, which is likely to play a vital role in cell morphology, motility, and other actin-related functions (Rivière *et al*., 2012). In neurons, ACTG1 is involved in shaping dendritic spines and synaptic structures (Schreiber *et al*., 2015), indicating its contribution to learning, memory, and neuroplasticity by facilitating synaptic remodelling. Variants in the *ACTG1* gene were observed in patients with Baraitser-Winter syndrome and agenesis of the corpus callosum and neuronal heterotopia (Vontell *et al*., 2019). Besides, several reports show variants in the *ACTG1* gene are associated with obsessive–compulsive disorder (Göbel *et al*., 2022) and autism spectrum disorder (Tuncay *et al*., 2022). Therefore, the processes associated with *ACTG1* in actin cytoskeletal dynamics may represent novel therapeutic targets for schizophrenia. However, the relationship between the *ACTG1* gene and the pathogenesis of schizophrenia requires further detailed elucidation.

Research indicates that antipsychotic medications can alter epigenomic patterns, including DNA methylation and histone modifications, affecting gene expression in the brain (Marques *et al*., 2025). Understanding the biological functions of epigenetics in the field of psychiatry would facilitate the development of new therapies for psychiatric disorders. In this study, we found amisulpride treatment can increase the protein level of ANP32E in the SH-SY5Y cells. The *ANP32E* gene encodes acidic nuclear phosphoprotein 32 family member E, which enables histone binding, acts as a histone chaperone, and assists in protein folding (Obri *et al*., 2014). It plays important roles in regulating chromatin, controlling gene expression, and helping the cell response to DNA damage (Obri *et al*., 2014). Gilda Stefanelli discovered that ANP32E plays a crucial role in regulating memory formation, transcription, and dendritic morphology by controlling steady-state H2A.Z binding in neurons (Stefanelli *et al*., 2021). Schizophrenia is increasingly recognised as a neurodevelopmental disorder that involves epigenetic factors (Yang *et al*., 2025b). We hypothesise that ANP32E impacts which neuronal genes are active or silenced during brain development by regulating chromatin structure through the eviction of H2A.Z. Taken together, we propose that amisulpride's action on ANP32E may be linked to the transcriptional regulation of target genes associated with histone chaperones, which provides potentially novel pathogenic mechanisms and treatment targets for psychiatric disorders.

The *IPO8* gene encodes importin-8, a nuclear transport belonging to the importin b family, which mediates the import of proteins into the nucleus (Görlich *et al*., 1997; Miyamoto *et al*., 2016). It plays crucial roles in nucleocytoplasmic trafficking, gene regulation, and signal transduction (Liang *et al*., 2013; Wei *et al*., 2014). A genetic study identified a single-nucleotide polymorphism in the *IPO8* gene that is associated with the brain systems responsible for eye movement, which are known to be impaired in psychotic disorders (Lencer *et al*., 2017). Notably, Nganou et al., demonstrated that *IPO8* knockdown was associated with defects in neuronal migration (Nganou *et al*., 2018). Numerous studies show that IPO8 is expressed in various tissues, including the adult brain, which is known to transport several proteins essential for brain development (Yao *et al*., 2008; Weinmann *et al*., 2009; Volpon *et al*., 2016). Based on the above evidence, we hypothesise that dysfunction of IPO8 could disrupt nucleocytoplasmic trafficking, resulting in abnormal transcriptional regulation of neuronal genes associated with schizophrenia. The up-regulation of IPO8 induced by amisulpride, as demonstrated in this study, may be relevant to the clinical efficacy of antipsychotic medications.

To summarise, our data suggest that amisulpride may influence the differential expression of genes and proteins related to the AP-1 transcription factor family, cytoskeleton, histone binding activity, intracellular trafficking of receptors, endocytosis of various macromolecules, and nuclear localisation signals. This understanding of the molecular mechanisms underlying the clinical effectiveness of amisulpride and the pathogenesis of schizophrenia opens up exciting possibilities for further research. The detailed transcriptomic and proteomic analysis of amisulpride treatment provides a comprehensive understanding of cellular response at the molecular level, sparking curiosity and the need for more in-depth studies in the field.

This study has a major limitation. SH-SY5Y cells may not be the ideal standalone model for schizophrenia research. Although SH-SY5Y cells are a human-derived neuroblastoma cell line widely used in neuroscience due to their catecholaminergic properties and their ability to differentiate into neuron-like cells, researchers often prefer to use primary neuronal cells, co-culture systems, and rodent models (Park *et al*., 2011; Shipley *et al*., 2016). These alternatives are more effective for investigating synaptic function, morphology, neurotoxicity, neurotransmitter release, and disease modelling. Consequently, the findings of this study should be interpreted with caution.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/neu.2025.10040.

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