Research Article

Hippocampal proteomic changes in a rat model of depression induced by dexamethasone

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Abstract

This study aimed to examine possible alterations of protein expression in the hippocampus of a rat model of depression induced by dexamethasone using the proteomic technique. The altered expression of several proteins has been found in a depressive group. The identified proteins play an important role in the cell signalling process, consisting of serotonergic, norepinephrinergic, dopaminergic, glutamatergic, and GABAergic receptors, the synaptic signalling associated proteins and the protein markers of GABAergic system. These results indicate the abnormality of signal transduction processes and the neurotransmitter dysfunction in depression. Moreover, up-regulation of beta-nerve growth factor, a neurotrophic factor involved in the survival of neurons, and down-regulation of amyloid-beta A4 precursor protein-binding family A member 1, a protein involved in the processing of the amyloid-beta precursor protein and signal transduction processes, were found in the depressive group. These findings reveal that the identified proteins in the hippocampus of the depressive model are important in the synaptic transmission process. It also shows synaptic dysfunction in depression. In summary, the identified proteins in this study by the proteomics technique could be used as the protein markers for further investigation.

Keywords: Proteomic analysis, Depression, Hippocampus, Dexamethasone, Synaptic dysfunction

Introduction

Depression is a severe neuropsychiatric disorder, which appears to be a multifactorial disease arising from genetic conjugated with environmental factors including stressful life events [1-2]. Neuropathological study in depressive patients reported that deficits in synaptic markers were found in the anterior cingulate, orbitofrontal, dorsolateral and prefrontal cortices [3]. Additionally, the alteration of neurogenesis factor was determined in the hippocampus of a rat depression model [4]. Dysregulations of serotonin (5-HT),

norepinephrine (NE), and dopamine (DA) neurotransmission have been reported to be a crucial part of the pathophysiology of depression [5]. A significant reduction of DA transporter (DAT) binding was examined in depressed patients with anhedonia [6]. The antagonists of glutamatergic N-methyl-D-aspartate (NMDA) receptor present antidepressant-like mechanisms in mice [7]. The reduction of glutamatergic neurons density was observed in the orbitofrontal cortex of patients with depression [8]. Decreased expression of NMDA receptor subunits (NR2A and NR2B) protein was detected in the prefrontal cortex of depressed patients [9]. Interestingly, reduced gamma aminobutyric acid (GABA) levels were found in plasma, cerebrospinal fluid (CSF) and cortex tissue of depressed patients [10-12]. $GABA_B$ receptor antagonists exhibited a potent of antidepressant-like effect in mice model of depression [13]. Previous studies showed that not only neurotransmission but neuroendocrine also involved in the development of the disease [5, 14-15]. The hypothalamic-pituitary-adrenal (HPA) axis is a major part of the neuroendocrine system that can stimulate the secretion of corticosterone [15]. The activation of the HPA axis response to stress exposure involved with glucocorticoid or corticosterone levels resulting in the development of depressive symptoms. Hippocampal volume reduction in depressive patients is vulnerable to stress and increased glucocorticoid levels [16]. Moreover, severe stress, depression, and anxiety-like behaviour were observed in animal exposure to glucocorticoid [17-1 8]. The animal models of depression revealed that a synthetic glucocorticoid, dexamethasone, can induce anhedonia and depression-like behaviors [19 -21]. The chronic high-dose of dexamethasone impairs long-term memory and motor coordination in mice [22]. In this study, dexamethasone was used to induce chronic stress, an animal model of depression. Several studies showed that approaches using proteomic techniques have recently arisen in search of a new explanation for the pathogenesis of neuropsychiatric disorders including anxiety, bipolar disorder, schizophrenia and depression [23 -25]. Using a proteomic approach, differentially expressed proteins depend on mood statuses that can be identified, which may help to understand the molecular pathway for the development of depression. In this study, we analyzed alterations in the rat hippocampal proteome following depression using LC-MS/MS. This study aimed to examine possible alterations of protein expression in the hippocampus of a rat depression model.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 180-220 g. were purchased from Nomura Siam International, Bangkok, Thailand. Animals were randomly assigned to depression and control groups. The control group (n=5), rats were injected subcutaneous (s.q.) with normal saline once a day for 4 weeks. The depressive group (n=5), rats were received 1.5 mg/kg dexamethasone once a day for 4 weeks. They were exposed a 12/12 h light/dark cycle at 24±1 ºC with a standard rodent diet and tap water available *ad libitum*. The anhedonia symptom, a major symptom of depression, was observed in the depressive group by the sucrose preference test (Kesyou et al., unpublished data). Rat brains were taken 24 h. after the last injection and were kept in -8 0 º C until the protein profile was studied. The experimental protocols were approved by the Animal Research Committee of Naresuan University, Phitsanulok, Thailand (permission 62 01 014).

Protein extraction and soluble protein digestion

The protein extraction was performed with a brief procedure as follows. The hippocampus tissues were homogenized in 5 mM Tris-HCl and 20 mM NaCl, pH 8.0. The homogenate tissue was centrifuged at 14,000 rpm for 10 min at 4 °C. The protein pellets were collected and re-homogenized in 50 mM Tris-HCl, 0.15 M NaCl, 0.1% SDS, 0.25% sodium deoxycholate and 1% protease inhibitor cocktail. Protein concentration was measured by bicinchoninic acid assay (Pierce, Rockford, IL., USA). An equal amount (µg) of individual proteins were pooled into control or depressive groups, based on protein concentration [26]. A total of protein sample was incubated with 10 mM dithiothreitol in 10 mM ammonium bicarbonate. The incubated protein was alkylated with 30 mM iodoacetamide (IAA) in 10 mM ammonium bicarbonate. The protein solution was then digested by 50 ng trypsin in 10 mM ammonium bicarbonate, and incubated overnight at 37°C. The digested proteins were purified by PureSpeed C18 tip and dried by vacuum evaporator. The dried peptide was dissolved in 0.1% formic acid (A) for further mass spectrometric analysis.

Liquid chromatography tandem-mass spectrometry analysis

Ten microliters of the digested peptide solutions were analyzed with Impact II UHR-TOF MS System (Bruker Daltonics Ltd., Germany) coupled to a nanoLC system: UltiMate 3000 LC System. Peptides were separated on a nanocolumn (PepSwift monolithic column 100 µm i.d. x 50 mm). Peptides were eluted with a linear gradient from 10-45% of B containing 80% acetonitrile in water containing 0.1% formic acid for 8.5 min at a flow rate of 1 µl/min. This was followed by a regeneration step at 90% B and an equilibration step at 1% B, one run took 20 min. Peptide fragment mass spectra were acquired in data-dependent AutoMS mode with selecting most abundant precursor ions in 3 second cycle for fragmentation. The range of the MS/MS scan was set to extend from 150 to 2200 *m/z*.

Proteins quantitation and identification

DeCyder MS Differential Analysis software (DeCyderMS, GE Healthcare) [27-28] was used to analyze the protein quantification. The DeCyder MS data were submitted to database searching over the Mascot software (Matrix Science, London, UK) [29]. The protein identification was searched against the National Center for Biotechnology Information (NCBI). The maximum value of each group was used to determine the presence or absence of each identified protein. The Uniprot retrieve/ID mapping tool (http://www.uniprot.org) was used to create file for protein identifications. Gene ontology annotation was analyzed by PANTHER (http://www.pantherdb.org) [30]. The protein interactions were described by The Search Tool for Interacting Chemicals (STITCH) (http://stitch.embl.de) [31].

Results

LC-MS/MS

LC-MS/MS analysis showed that total 5,528 proteins were observed in the hippocampus of the control group, which 3,549 proteins were differentially expressed relative to the depressive group. Uniquely expressed protein in the control group may reveal the down-regulated expression of these proteins in the depressive group depend on the detection capability of LC-MS/MS method. Five thousand and five hundred twenty-six proteins were identified in the depressive group. Three thousand and five hundred forty-seven proteins were uniquely expressed, which may represent the up-regulated expression relative to control. We found that 1,979 proteins were co-expressed during control and depressive groups. A protein was considered differentially expressed if the

fold change was ≥ ±1.2 [26]. One thousand and three hundred sixty-five proteins were selected for further interaction analysis.

Functional analysis of identified proteins

The biological processes of these identified proteins were categorized using PANTHER db according to function in cellular process, metabolic process, biological regulation, the term of response to stimulus, cellular component organization or biogenesis, localization, signalling, multicellular organismal process, developmental process, immune system process, locomotion, and others. **Figure 1A** shows differentially expressed protein in the control group. **Figure 1B** represents uniquely expressed proteins in the depressive group. In addition, 1,365 overlapping proteins during control and depressive groups were also analyzed by PANTHER db as shown in **Figure 1C**.

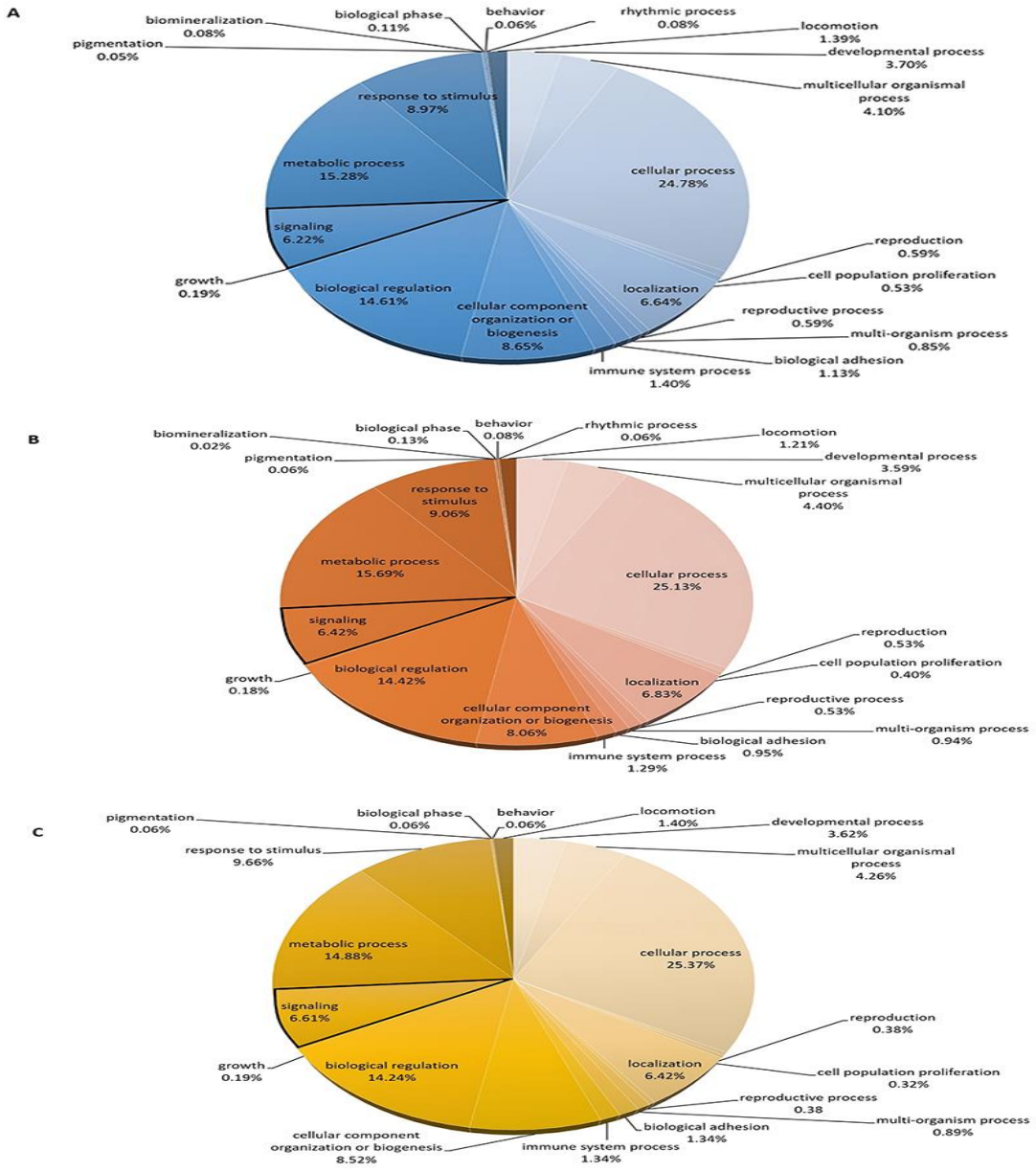

Figure 1 The biological processes were analyzed by PANTHER db. A; control group, B; depressive group, and C; co-expressed protein.

We focused on biological process especially the cell signalling process, which may play an important role in the central nervous system. The proteomic profile of both control and depressive groups were described in **Table 1**. We found that uniquely expressed proteins were altered according to depressive status. The altered expression of neurotransmission receptors was found. The up-regulation of 5-hydroxytryptamine receptor 1D, acetylcholine receptor subunit gamma, adenosine receptor A1, alpha-2B adrenergic receptor, D(1 B) dopamine receptor, D(3) dopamine receptor, GABA θ subunit, GABA $A_{\gamma 2}$ long isoform, GABA Aδ, GABA $A_{\gamma 1}$, GABA $A_{\rho 1}$, glutamate receptor (1, 2, 3), glutamate receptor ionotropic kainite (1, 2, 3, 5), glycine receptor subunit α-3, metabotropic glutamate receptor 7, neuronal acetylcholine receptor subunit (α-2, β-3) was observed, while the down-regulation of 5-hydroxytryptamine receptor (2B, 5B), adenosine receptor A2a, beta-1 adrenergic receptor, D(2) dopamine receptor, GABA Aθ, glutamate receptor ionotropic(δ-1, δ-2, NMDA 2A, NMDA 2C), glutamate receptor, metabotropic 1, isoform CRA_b, glycine receptor subunit beta, metabotropic glutamate receptor 3, muscarinic acetylcholine receptor M3, was found. Not only the changes of neurotransmission receptor were found but the synaptic signalling associated proteins were also observed. Increased expression of corticoliberin, excitatory amino acid transporter 3, neuroendocrine protein 7B2, neuroligin-2, neuroligin-3, neurotrophin-4, otoferlin, prepronociceptin, sodium channel protein type 10 subunit alpha, sodium channel protein type 11 subunit alpha, solute carrier family 12 member 4, solute carrier family 7 member 13, synapsin-1, synapsin-3, synaptosomal-associated protein, synaptosomal-associated protein 23, synaptotagmin-2, synaptotagmin XI, syntaxin-binding protein 1, transcription factor 7-like 2, tyrosine 3-monooxygenase was identified. On the other hand, the reduced expression of annexin A1, complexin 4, gamma-aminobutyric acid receptor-associated protein-like 2, multiple C2 and transmembrane domain-containing protein 1, neurotrophin-3, NMDA receptor synaptonuclear-signaling and neuronal migration factor, protein cornichon homolog 3, protein unc-13 homolog B, putative sodium-coupled neutral amino acid transporter 7, receptor-type tyrosine-protein phosphatase N2, sodium- and chloride-dependent glycine transporter 1, sodium channel protein type 1 subunit alpha, synaptotagmin-3, voltage-dependent calcium channel gamma-8 subunit, voltage-dependent L-type calcium channel subunit beta-4 was found. Furthermore, the alteration of other functioning proteins such as arginine and glutamate-rich protein 1, calbindin, calretinin, amyloid-beta A4 precursor protein-binding family A member 1, guanine nucleotide-binding protein G (olf) subunit alpha, mitochondrial glutamate carrier 2 was found.

Table 1 Uniquely expressed protein in the rat hippocampus.

Accession No.	Protein name	Gene name	Intensity (log2)
Depressive group			
P28565	5-hydroxytryptamine receptor 1D	Htr1d	19.1190
P18916	Acetylcholine receptor subunit gamma	Chrng	16.9792
P25099	Adenosine receptor A1	Adora1	15.6320
P19328	Alpha-2B adrenergic receptor	Adra2b	18.2497
Q5BJT0	Arginine and glutamate-rich protein 1	Arglu1	18.0206
P07171	Calbindin	Calb1	12.4672
P07171	Calbindin	Calb1	12.4672
P01143	Corticoliberin	Crh	13.7686
P25115	D(1B) dopamine receptor	Drd5	16.6597
P19020	D(3) dopamine receptor	Drd3	15.1296
Q5XIX0	DnaJ homolog subfamily C member 14	Dnajc14	17.6867
P51907	Excitatory amino acid transporter 3	Slc1a1	13.6428
D4AD36	FCH and double SH3 domains 1	Fchsd1	16.1010
Q91ZM7	GABA theta subunit	Gabrq	16.5987
Q6PW52	GABA-A gamma2 long isoform	Gabrg2	17.3558
P18506	Gamma-aminobutyric acid receptor subunit delta	Gabrd	18.1112
P23574	Gamma-aminobutyric acid receptor subunit gamma-1	Gabrg1	21.0027
P50573	Gamma-aminobutyric acid receptor subunit rho-3	Gabrr3	17.6239
P19490	Glutamate receptor 1	Gria1	14.0181
P19491	Glutamate receptor 2	Gria2	19.0552
G3V6Z5	Glutamate receptor 3	Gria3	17.0771
P22756	Glutamate receptor ionotropic, kainate 1	Grik1	18.0113
F1M855	Glutamate receptor ionotropic, kainate 2	Grik2	19.6956
P42264	Glutamate receptor ionotropic, kainate 3	Grik3	16.3285
Q63273	Glutamate receptor ionotropic, kainate 5	Grik5	18.1761
P24524	Glycine receptor subunit alpha-3	Glra3	17.9937
Q63803	Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas	Gnas	18.4502
P35400	Metabotropic glutamate receptor 7	Grm7	17.5863
P27682	Neuroendocrine protein 7B2	Scg5	18.2800
F1LQ41	Neuroligin-2	Nlgn2	16.3668
D3ZDC0	Neuroligin-3	Nlgn3	14.4247
P12389	Neuronal acetylcholine receptor subunit alpha-2	Chrna2	19.0278
P12391	Neuronal acetylcholine receptor subunit beta-3	Chrnb3	18.9638
P34131	Neurotrophin-4	Ntf4	17.9685
D4A026	Olfactory receptor	Olr519	20.0231
D3ZB83	Olfactory receptor 1576	Olr1576	17.7377
Q9ERC5	Otoferlin	Otof	16.7350
Q62923	Prepronociceptin	Pnoc	17.3738
P63012	Ras-related protein Rab-3A	Rab3a	18.8427
Q63259	Receptor-type tyrosine-protein phosphatase-like N	Ptprn	19.1953
Q62968	Sodium channel protein type 10 subunit alpha	Scn10a	13.7385
O88457	Sodium channel protein type 11 subunit alpha	Scn11a	18.9608
Q63632	Solute carrier family 12 member 4	Slc12a4	18.8099

Table 1 Uniquely expressed protein in the rat hippocampus. (continued)

Accession No.	Protein name	Gene name	Intensity (log2)
Depressive group			
Q5RKI7	Solute carrier family 7 member 13	Slc7a13	17.8667
P09951	Synapsin-1	Syn1	19.8250
O70441	Synapsin-3	Syn3	14.8269
D4A5W9	Synaptosomal-associated protein	Snap25	17.3404
O70377	Synaptosomal-associated protein 23	Snap23	15.7266
P29101	Synaptotagmin-2	Syt2	20.4248
Q505J5	Synaptotagmin XI	Syt11	14.9028
P61765	Syntaxin-binding protein 1	Stxbp1	20.142
D4A8X6	Transcription factor 7-like 2	Tcf7l2	14.2096
P04177	Tyrosine 3-monooxygenase	Th	18.2182
P30994	5-hydroxytryptamine receptor 2B	Htr2b	10.2324
P35365	5-hydroxytryptamine receptor 5B	Htr5b	14.1685
P30543	Adenosine receptor A2a	Adora2a	15.4145
O35430	Amyloid-beta A4 precursor protein-binding family A member 1	Apba1	14.5616
P07150	Annexin A1	Anxa1	11.3071
P18090	Beta-1 adrenergic receptor	Adrb1	16.7927
P47728	Calretinin	Calb2	18.1808
D3ZM85	Complexin 4	Cplx4	15.9637
P61169	D(2) dopamine receptor	Drd2	18.5736
Q62696	Disks large homolog 1	Dlg1	19.4231
P60522	Gamma-aminobutyric acid receptor-associated protein-like 2	Gabarapl2	16.7996
G3V875	Gamma-aminobutyric acid type A receptor theta subunit	Gabrq	17.8686
Q62640	Glutamate receptor ionotropic, delta-1	Grid1	17.9629
F1LXB6	Glutamate receptor ionotropic, delta-2	Grid2	13.7036
G3V9C5	Glutamate receptor ionotropic, NMDA 2A	Grin2a	12.8623
Q00961	Glutamate receptor ionotropic, NMDA 2C	Grin2c	13.4807
G3V7U1	Glutamate receptor, metabotropic 1, isoform CRA_b	Grm1	18.6912
P20781	Glycine receptor subunit beta	Glrb	18.4208
P38406	Guanine nucleotide-binding protein G(olf) subunit alpha	Gnal	13.1408
P31422	Metabotropic glutamate receptor 3	Grm3	15.1593
Q505J6	Mitochondrial glutamate carrier 2	Slc25a18	19.8768
D4ABL6	Multiple C2 and transmembrane domain-containing protein 1	Mctp1	17.0103
P08483	Muscarinic acetylcholine receptor M3	Chrm3	17.8498
P18280	Neurotrophin-3	Ntf3	21.2746
D3ZVR5	NMDA receptor synaptonuclear-signaling and neuronal migration factor	Nsmf	15.3422
D4A3V1	Olfactory receptor	Olr1622	16.3867
Q9ES40	PRA1 family protein 3	Arl6ip5	13.9008
D0Q0Y7	Protein cornichon homolog 3	Cnih3	19.0309
Q62769	Protein unc-13 homolog B	Unc13b	12.2126
Q6JWR2	Putative sodium-coupled neutral amino acid transporter 7	Slc38a7	12.733
Q63475	Receptor-type tyrosine-protein phosphatase N2	Ptprn2	14.5141
D3ZPJ0	Shisa family member 7	Shisa7	21.7792
P28572	Sodium- and chloride-dependent glycine transporter 1	Slc6a9	16.2638

Table 1 Uniquely expressed protein in the rat hippocampus. (continued)

Accession No.	Protein name	Gene name	Intensity (log2)
Depressive group			
P04774	Sodium channel protein type 1 subunit alpha	Scn1a	14.4327
P40748	Synaptotagmin-3	Syt3	18.9182
Q8VHW5	Voltage-dependent calcium channel gamma-8 subunit	Cacng8	16.2011
D4A055	Voltage-dependent L-type calcium channel subunit beta-4	Cacnb4	12.7526

The alteration of co-expressed proteins compared between depressive and control groups was presented in the Heat-map (**Figure 2**). Twenty-one overlapping expressed proteins were found. The reduction of 7 co-expressed proteins including 5-hydroxytryptamine receptor 6, gamma-aminobutyric acid receptor subunit rho-2, muscarinic acetylcholine receptor M1, protein lin-7 homolog C, synaptosomal-associated protein 25, synaptotagmin-17, and dishevelled-binding antagonist of beta-catenin 1 was found in the depressive group relative to control. Additionally, up-regulation of 14 proteins as follows beta-nerve growth factor, gamma-aminobutyric acid receptor subunit alpha-2, ionotropic glutamate receptor kainate 4, glycine receptor alpha 4, Sn1-specific diacylglycerol lipase alpha, sodium channel protein type 3 subunit alpha, synapsin-2, synaptotagmin-5, dickkopf WNT-signaling pathway inhibitor 2, segment polarity protein dishevelled homolog DVL-1, protein phosphatase 1B, voltage-dependent calcium channel gamma-4 subunit, protein Wnt7a, protein Wnt10a, was observed in the depressive group when compared to control.

STITCH analysis was used to describe the interaction of co-expressed proteins underlying the cell signalling process (**Figure 3**). This study dexamethasone, a synthetic glucocorticoid agonist, was used to induce chronic stress, a model of depression. The induction of this depressive model may be associated with the hippocampal dysfunction through the several neurotransmissions as follows serotonin, acetylcholine, GABA, and glycine. Dexamethasone causes a change in corticosterone hormone resulting in neurotransmission deficits and disability of synaptic transmission. However, interaction results from STITCH have shown no detection of dexamethasone effect on beta-nerve growth factor, glutamate receptor ionotropic, kainate 4, Protein lin-7 homolog C, Sn1-specific diacylglycerol lipase alpha, sodium channel protein type 3 subunit alpha, synaptotagmin-17, dickkopf WNT-signaling pathway inhibitor 2, segment polarity protein dishevelled homolog DVL-1, protein Wnt, protein phosphatase 1B, voltage-dependent calcium channel gamma-4 subunit, protein Wnt, dishevelled-binding antagonist of beta-catenin 1.

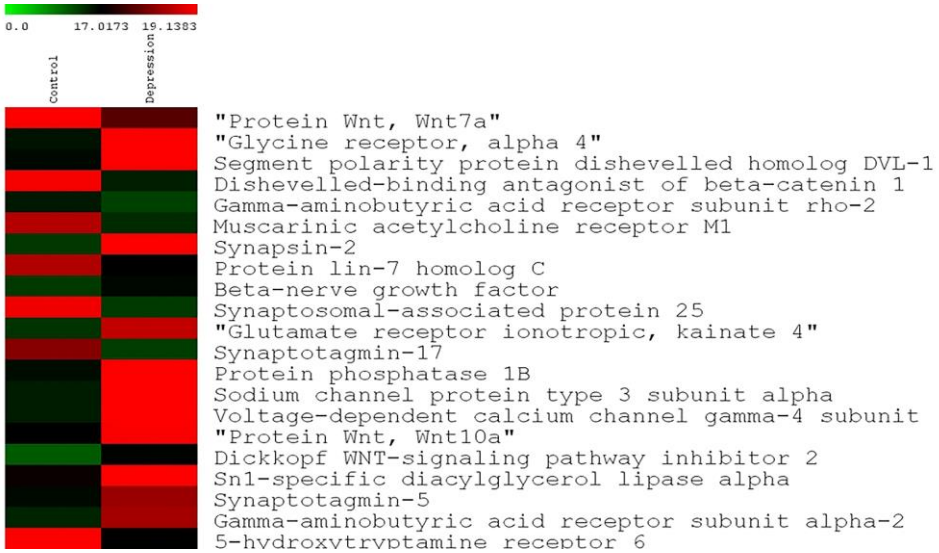

Figure 2 Heat-map presentation of 21 proteins according to the cell signalling process (absent in green, low in dark green, and highest in red).

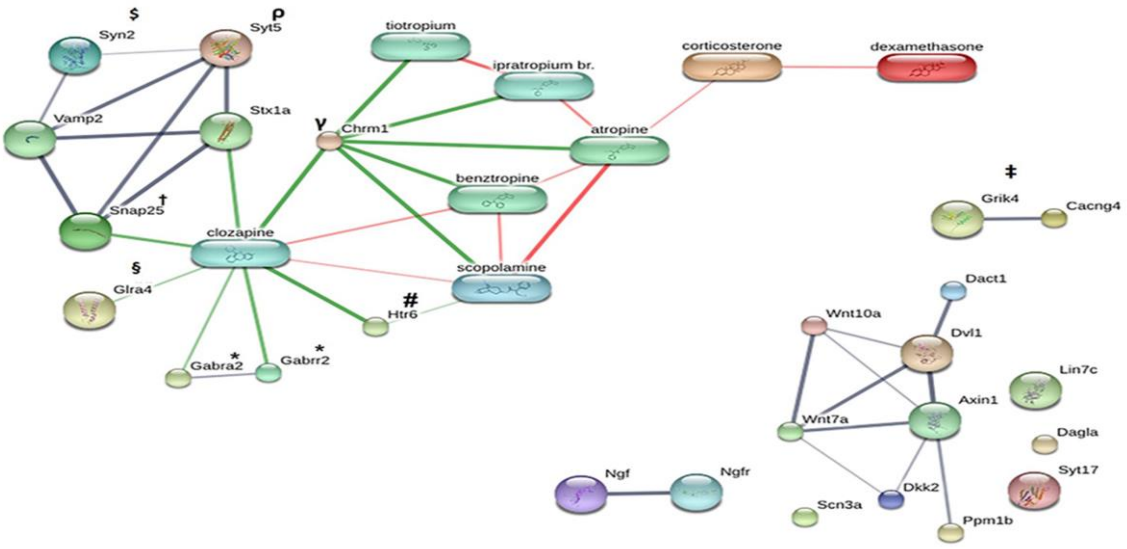

Figure 3 STITCH analysis shows the interaction between protein and small molecule, 5-hydroxytryptamine receptor 6 (Htr6), Beta-nerve growth factor (Ngf), Gamma-aminobutyric acid receptor subunit alpha-2 (Gabra2), Gamma-aminobutyric acid receptor subunit rho-2 (Gabrr2), Ionotropic glutamate receptor kainate 4 (Grik4), Glycine receptor alpha 4 (Glra4), Muscarinic acetylcholine receptor M1 (Chrm1), Protein lin-7 homolog C (Lin7c), Sn1-specific diacylglycerol lipase alpha (Dagla), Sodium channel protein type 3 subunit alpha (Scn3a), Synapsin-2 (Syn2), Synaptosomal-associated protein 25 (Snap25), Synaptotagmin-17 (Syt17), Synaptotagmin-5 (Syt5), Dickkopf WNT-signaling pathway inhibitor 2 (Dkk2), Segment polarity protein dishevelled homolog DVL-1 (Dvl1), Protein phosphatase 1B (Ppm1b), Voltage-dependent calcium channel gamma-4 subunit (Cacng4), Protein Wnt (Wnt7a), Protein Wnt (Wnt10a), Dishevelled-binding antagonist of beta-catenin 1 (Dact1).

* represent GABA receptor, † represent synaptosomal-associated protein 25, § represent glycine receptor, ‡ represent glutamate receptor, ρ represent synaptotagmin-5, $ represent synapsin-2, γ represent acetylcholine receptor, and # represent serotonin receptor.

Discussions and Conclusions

Our study represents the systematic identification and quantification of the proteomic approach in the pathophysiology of depression. We found that the identified proteins in this study play an important role in the central nervous system, particularly neurotransmission receptors, neurotransmission transporters, voltage-dependent channels, and synaptosomal associated proteins. According to the previous report, a significant decrease in the synaptosomal associated protein was found in several psychological diseases including bipolar, schizophrenia, and depression [32]. The reduction of synaptosomal-associated protein 25 (SNAP-25) was found in the depressive group relative to control. SNAP-25 is a soluble N-ethylmaleimide-sensitive factor activating protein receptor found in the plasma membrane. This protein is important in the regulation of voltage-gated calcium channels and transmission of neurotransmitters between nerve cells. In line with the previous study, the SNAP-25 gene polymorphism correlates with the depression score detected by the Temperament and Character Inventory (TCI) was found in women with fibromyalgia syndrome [33]. The up-regulation of synapsin 2, a neuronal phosphoprotein, was found in the depressive group relative to control. Synapsins present as a presynaptic protein due to their abundance on synaptic vesicles and their contribution to synaptic communication. A previous study has shown the association of synapsin 2 in the formation and maintenance of synapses in hippocampal neurons [34]. We also found increased expression of synaptotagmin-5 (SYT5), a calcium-binding synaptic vesicle protein, in the depressive group compared to control. Mutation in synaptotagmin 1 (SYT1) gene reduced synaptic vesicle fusion kinetics [35]. SYT3 protein also influenced in the impaired memory [36]. Our finding confirms the impaired neuronal transmission in the hippocampus of the depressive group. Furthermore, the disability of neurotransmission was found in this study is consistent with previous reports. The deficits of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) neurotransmission were observed in a transgenic mouse model of depression [5]. Up-regulated expression of alpha2A-adrenoceptors and serotonin receptor genes was examined in the depressed suicide victims [37]. Down-regulation of metabotropic glutamate receptor 5 [38] and NMDA receptor subunits (NR2A and NR2B) [9] were determined in depressed patients. Moreover, the alterations of GABAergic receptor gene expression have been reported in depression. Increased expression of GABA A receptor (alpha 1, alpha 3 and alpha 4 subunits) genes were found in the depressed suicide victims [39]. The reduction of GABA A_{ρ_1} receptor was found in depressed suicides [40]. STITCH analysis shows the influence of corticosterone on dysregulation of 5-HT6, GABA receptor alpha-2, GABA receptor rho-2, glycine receptor alpha 4, muscarinic acetylcholine receptor M1, synapsin-2, synaptosomal-associated protein 25, and synaptotagmin-5. This finding indicates the effect of the hypothalamic-pituitary-adrenal (HPA) axis, a major part of the neuroendocrine system on the

synaptic neuronal activities in the development of depression. Moreover, the changes of calbindin and calretinin, calcium-binding proteins, that are GABAergic interneuron markers. Previous studies reported that the deficit of calbindin but not calretinin was found in the hippocampus of the schizophrenia post-mortem brain [41] and a rat model [42]. The deficit effects of the GABAergic biomarkers in conjunction with GABA receptor protein alterations indicate the critical role of the GABAergic neurotransmission in the development of depression. Interestingly, reduced amyloid-beta A4 precursor protein-binding family A member 1 (APBA1) was identified in the depressive group relative to control. APBA1 stabilizes amyloid precursor protein, which is believed to be involved in signal transduction processes in Alzheimer's disease [43]. Expression levels of 5-HT6 receptor and 5-HT7 receptor are closely correlated with APBA1 expression in the hippocampus of patients with depression [44]. Thus, the evidence that APBA1 reduction suggests loss of transduction signalling in depression. The present study also shows the increased expression of beta-nerve growth factor (β-NGF) in the depressive group, which is consistent with previous studies. Altered levels of NGF have been reported to influence the disease severity according to the Hamilton Depression Rating Scale (HAM-D) 17-item. Increased levels of serum NGF were found in patients with depression [45-46]. In contrast, previous studies reported a significant reduction in peripheral NGF levels [47-48]. NGF has been found to play important roles in the survival of sympathetic and some sensory and central cholinergic neurons. Therefore, the consequence of NGF activation underlying the disease severity should be further confirmed for clarification of depressive mechanisms. These findings indicate that identified proteins in rat hippocampus of chronic mild stress an animal model of depression is important in the cell signalling process, particularly synaptic transmission. It also pointed out synaptic dysfunction in depression.

In conclusion, this study shows the alteration of several proteins which are essential for synaptic transmission in the hippocampus including neurotransmission receptors, neurotransmission transporters, voltage-dependent channels, and synaptosomal associated proteins underlying the pathophysiology of depression. This finding indicates a deficiency in the neuronal signal transduction processes, the impairment of the neuronal functions, and the neurotransmitter dysfunction within the central nervous system in depression. These differentially expressed proteins may be potential protein markers for diagnostic and therapeutic targets of depression. The expression of identified proteins should be confirmed using other methods such as the western blot technique. The proteomic analysis should be confirmed in other animal models of depression and depressed patients for a clear understanding of the disease mechanisms.

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