Research Article

Proteomic analysis of frontal cortex proteins in a rat model of depression induced by dexamethasone

Siriluk Veerasakul[1], Korakod Bandasak[2], Plaiyfah Janthueng[3,4], Sittiruk Roytrakul[5], Samur Thanoi[3,4] and Sutisa Nudmamud-Thanoi[3,4*]

[1] Department of Occupational Health and Safety, School of Public Health, Walailak University, Nakhon Si Thammarat 80160

[2] Faculty of Medical Science, Naresuan University, Phitsanulok, 65000

[3] Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok 65000

[4] Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000

[5] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani 12120

* Corresponding author: sutisat@nu.ac.th

Naresuan Phayao J. 2021;14(1)**:** 3-15.

Received; 6 October 2020; Revised: 25 November 2020; Accepted: 20 April 2021

Abstract

This study was designed to characterize the identified proteins in the frontal cortex of a rat model of depression induced by dexamethasone using proteomic technique. Identified proteins were analyzed according to the biological function. The identified proteins were categorized in the response to the cellular process, metabolic process, and others including cell-cell signaling, which are associated with the brain functions. Results showed that uniquely expressed proteins in control and depressive groups reveal the function of monoamine, glutamate and GABA receptors and appear to be a part of the protein transporter, the regulating protein of cell communication and synapse. In addition, a decrease of the co-expressed proteins was found in the depressive group when compared to the control group. These proteins are associated with the function of the dopamine neurotransmitter system. The results of this study suggest that the associated proteins in synaptic transmission, particularly neurotransmitter receptors, regulating proteins of cell communication and synapse play an important role in the pathophysiology of depression. Furthermore, the identified proteins in this study may be used as a biological marker in the study of frontal cortex function to further clarify the mechanism for depression. That may lead to appropriate treatment.

Keywords: Proteomics, Depression, Frontal cortex, Dexamethasone

Introduction

Depression is the most common mental health disorder. The clinical symptoms are characterized by low mood, low self-esteem, loss of pleasure, thoughts of death and suicide. Depression results from a complex interaction of social, psychological, and biological factors [1]. There have been several hypotheses for the pathogenesis of depression, including the alteration of neurotransmitters [2-4] and disability of the hypothalamic-pituitary-adrenal (HPA) axis [5]. The efficacy of antidepressants suggests that an abnormality in serotonergic and noradrenergic neurotransmissions is the core of the pathophysiology of depression. An imbalance in serotonin (5-HT), norepinephrine (NE), and dopamine (DA) neurotransmission have been reported to underlie the pathophysiology of depression [6]. The metabolite of 5-HT appears to be low in cerebrospinal fluid (CSF) of patients with depression [7]. The functioning study reported that the alteration of α_{2A}-adrenoceptors associate with the release of NE [4]. A significant decrease of DA transporter (DAT) binding was observed in depressed patients with anhedonia relative to healthy subjects [8]. Furthermore, the glutamatergic system is implicated in the pathophysiology of depression. N-methyl-D-aspartate (NMDA) receptor antagonists administration is reported to present antidepressant-like mechanisms in mice [9]. A post-mortem study showed that the density of glutamatergic neurons was reduced in the orbitofrontal cortex of patients with depression [10]. The expression of NMDA receptor subunits (NR2A and NR2B) protein were decreased in the prefrontal cortex of depressed patients [11]. In addition, previous studies have reported that the therapeutic effects of used monoaminergic antidepressants are involved in the alterations of GABAergic transmission [3]. $GABA_B$ receptor antagonists exhibited a potent of antidepressant-like effect in $GABA_{B(1)}$ receptor knockout mice [12]. The reductions of GABA levels were found in plasma, CSF and cortical cortex of depressed patients [13-15]. Interestingly, not only neurotransmission hypotheses but the consequence of stress hypothesis is also involved in depression. Extreme exposure to stress can lead to the development of depressive symptoms that may be involved with the HPA axis function, which is a major part of the neuroendocrine system. The activation of the HPA axis can stimulate the secretion of corticoids (cortisol in humans and corticosterone in rodents) [5]. It is well known that this hormone regulates neuronal survival, neuronal excitability, neurogenesis and memory acquisition [16]. Increased levels of the glucocorticoid were found in depressed patients with the exhibition of the HPA axis hyperactivity [17]. Moreover, orally glucocorticoid treatment can induce severe stress, depression, and anxiety-like behaviour in animal [18, 19]. Several studies have reportd that anhedonia and depression-like behaviors were found after exposure to dexamethasone, a synthetic glucocorticoid receptor agonist [20-22]. In the present study, dexamethasone was used to induce the animal model of depression.

The proteomic approach was used to investigate global profiles of protein expression. It is widely used to examine the sets of differentially expressed protein for different mood statuses and usually applied to identify in comparisons of two or more different states [23, 24]. Using this technique may be established an understanding of the pathophysiology of depression. Therefore, this study aimed to characterize identified proteins in dexamethasone induced depression in rats using proteomic technique.

Materials and Methods

Animals

Male Sprague-Dawley rats from Nomura Siam International, Bangkok, Thailand weighing 180-220 g were divided into 2 groups. Animals were injected subcutaneous (s.q.) with normal saline or dexamethasone one injection per day for 4 weeks. Control group (n=5), rats were received normal saline. Depressive group (n=5), rats were received 1.5 mg/kg dexamethasone. Animals were housed under a 12/12 h light/dark cycle at 24±1 ºC with food and water available *ad libitum* in their home cage. Rat brains were taken 24 h after the last injection and were kept in -80 ºC until protein profile study. All experiments were approved by the Animal Research Committee of Naresuan University, Phitsanulok, Thailand (permission 62 01 014).

Protein extraction and protein digestion

The frontal cortex 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 ºC. 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 (P8340, Sigma-Aldrich). Protein concentration was measured by bicinchoninic acid assay (Pierce, Rockford, IL., USA).

Equal amount (μg) of individual proteins were pooled into control or depressive groups for LCMS/MS [25,26]. A total of protein sample was incubated with 10 mM dithiothreitol (DTT) in 10 mM ammonium bicarbonate at 56 ºC for 1 h. After that, iodoacetamide (IAA) (30 mM DTT in 10 ammonium bicarbonate) was mixed and stilled room temperature for 1 h. Fifty nanograms trypsin in 10 mM ammonium bicarbonate were added into the mixture. The protein sample was incubated overnight at 37 ºC. 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

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. The gradient elution was treated with 10-45% B (80% acetonitrile in water containing 0.1% formic acid) for 8.5 min at a flow rate of 1 μl/min, including a regeneration step at 90% B and an equilibration step at 1% B, one run took 20 min. The MS data were acquired using the following set up: data-dependent AutoMS mode with selecting most abundant precursor ions in 3 second cycle for fragmentation. The range of the MS/MS scan was extended from 150 to 2200 *m/z*.

Proteins quantitation and identification

The protein quantification was analyzed by DeCyder MS Differential Analysis software (DeCyderMS, GE Healthcare) [27, 28]. The analyzed MS/MS data from DeCyder MS were submitted to database searching through the Mascot software (Matrix Science, London, UK) [29]. Database interrogation was taxonomy, mass values (monoisotopic); protein mass (unrestricted); peptide mass tolerance (1.2 Da); fragment mass tolerance (±0.6 Da), peptide charge state (1+, 2+ and 3+) and max missed cleavages. The data were searched against the National Center for Biotechnology Information (NCBI). The files of protein identifications were created using the Uniprot retrieve/ID mapping tool (http://www.uniprot.org). The protein bioinformatics especially biological process was

analyzed by PANTHER (http://www.pantherdb.org) [30]. The identified proteins were simultaneously submitted to the Search Tool for Interacting Chemicals (STITCH) (http://stitch.embl.de) in order to understanding of the interactions between proteins and small molecules [31].

Results

LC-MS/MS

The result showed that 4,635 proteins were analyzed by LC-MS/MS analysis, 4,203 proteins of which were expressed depending on group difference. Two thousand and three hundred thirteen differentially expressed proteins were found in the frontal cortex of the control group, which may predict the down-regulated expression of these proteins in the depressive group with respect to the detection capability of this method. However, 1,890 differentially expressed proteins were observed in the frontal cortex of the depressive group, which may represent the up-regulated expression relative to control. We found that 432 proteins co-expressed during control and depressive groups.

Ontology of identified proteins

The biological functions of identified proteins were categorized using PANTHER db according to their biological process. The differentially expressed proteins in control and depressive groups are involved in the response to the cellular process, metabolic process, biological regulation, cellular component biogenesis, the term of response to a stimulus, localization, signaling, multicellular organismal process, developmental process, biological adhesion, immune system process, multi-organism process, locomotion, and others as shown in figure 1A and 1B, respectively. Co-expressed proteins during control and depression were classified by the biological process as shown in figure 1C. We focused on identifying proteins linked to specific functions carried out at the neurotransmitter role in the central nervous system. Interestingly, the cell-cell signaling process may indicate the function of the neurotransmission within the frontal cortex. Uniquely expressed proteins in both depressive and control groups with specifically in the cell-cell signaling process were presented in table 1. LC-MS/MS analysis showed the increased expression of neurotransmitter receptors including 5-HT receptor 1F, alpha-2C adrenergic receptor, GABA B receptor 1, GABA A receptor (alpha 6 and p_i), glutamate receptor 2, glutamate delta-1 receptor, glutamate receptor ionotropic (NMDA 2D, NMDA 3A), metabotropic glutamate receptor 7 in depressive group, while a decreased expression of 5-HT receptor 5B, adrenergic receptor (alpha 1d, alpha 2a, and beta 1), GABA A receptor (alpha 2 and gamma 6), metabotropic glutamate receptor 1, muscarinic acetylcholine receptor M5 was observed in depressive group relative to control. The expression of Glutamate decarboxylase 2 or GAD_{65}, an enzyme in GABA synthesis, was increased in depressive group compared to control. In addition, the expression of transporter associated proteins such as sodium- and chloride-dependent glycine transporter 1 and solute carrier family 12 member 4 were decreased in depression relative to control. Increased expression of sodium channel protein, sodium channel protein type 11 subunit alpha, voltage-dependent calcium channel gamma-3 subunit, and voltage-dependent L-type calcium channel subunit beta-3 was examined, while the reduction of sodium channel protein type 10 subunit alpha, voltage-dependent calcium channel gamma-4 subunit, and voltage-dependent T-type calcium channel subunit alpha-1H was determined in depressive rats compared to controls. Almost all the synaptosomal-associated proteins that are identified in this study, revealed a decrease of expression in the depressive group excluding synaptotagmin-1 and syntaxin-4.

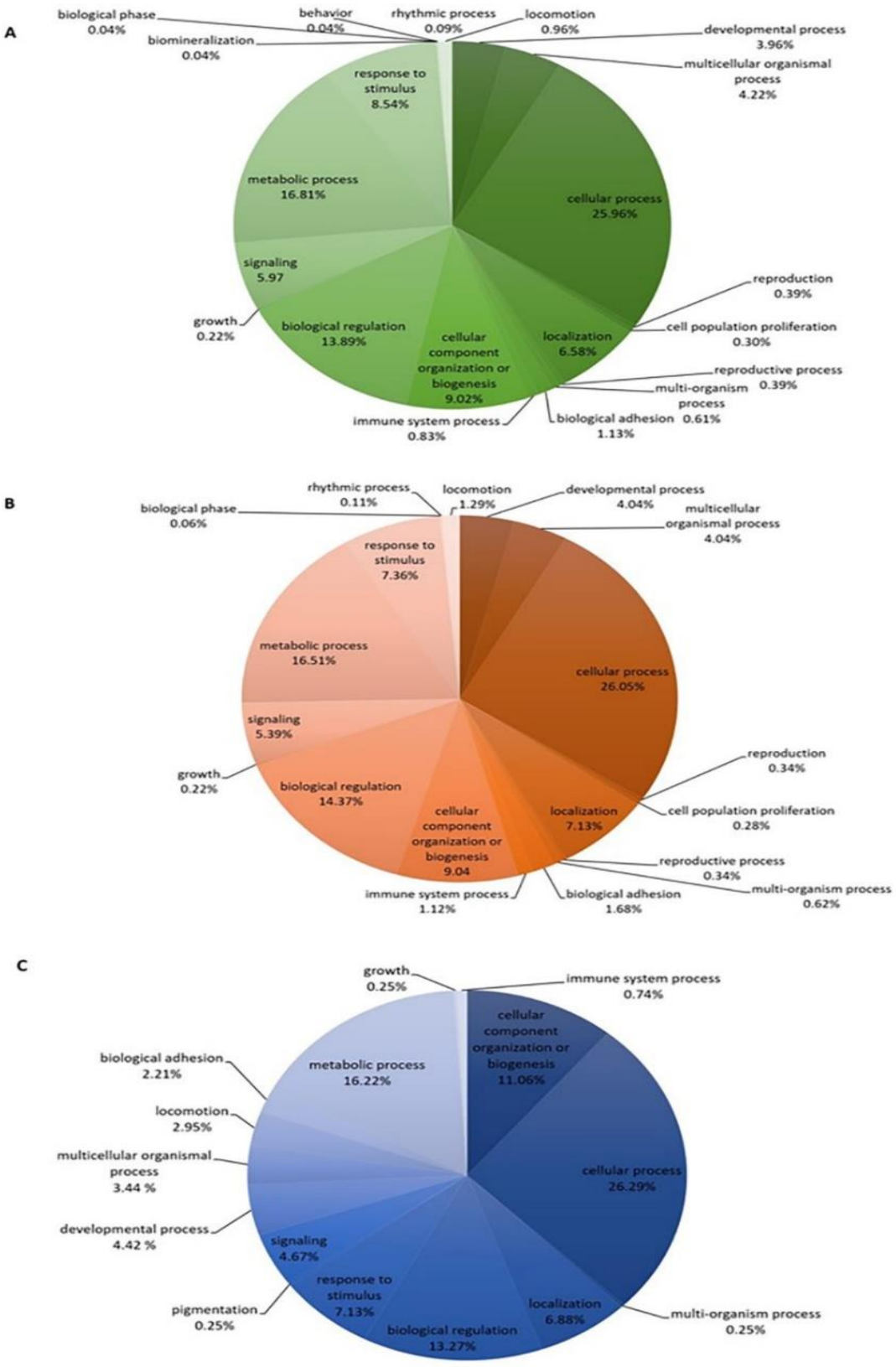

Figure 1 The biological functions underlying biological processes were categorized by PANTHER db. A) represents the biological process in the control group. B) shows functioning identification in the depressive group. C) reveals the biological process of overlapping protein expression during the control and the depressive group.

Table 1 Differentially expressed proteins in the rat frontal cortex.

Accession No.	Protein name	Gene name	Intensity (log2)
Depressive group			
P30940	5-hydroxytryptamine receptor 1F	Htr1f	17.7656
P22086	Alpha-2C adrenergic receptor	Adra2c	15.0077
A0A0G2JZW4	APC2, WNT-signaling pathway regulator	Apc2	14.0916
Q6Q0N0	Calsyntenin-1	Clstn1	16.2545
P31016	Disks large homolog 4	Dlg4	15.8949
Q6MFX8	Gamma-aminobutyric acid (GABA) B receptor 1	Gabbr1	15.7140
G3V6G3	Gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 6, isoform CRA_a	Gabra6	14.2899
F1LM27	Gamma-aminobutyric acid receptor subunit pi	Gabrp	16.5298
Q05683	Glutamate decarboxylase 2	Gad2	13.9918
F1LNE4	Glutamate receptor 2	Gria2	13.6390
Q62640	Glutamate receptor ionotropic, delta-1	Grid1	15.1993
Q62645	Glutamate receptor ionotropic, NMDA 2D	Grin2d	20.6127
Q9R1M7	Glutamate receptor ionotropic, NMDA 3A	Grin3a	16.4771
F1LZS5	Metabotropic glutamate receptor 7	Grm7	10.7536
D3ZDC0	Neuroligin-3	Nlgn3	15.5564
P12390	Neuronal acetylcholine receptor subunit beta-2	Chrnb2	16.7487
P0C0P7	Neuropeptide S	Nps	16.5723
Q9JIR0	Peripheral-type benzodiazepine receptor-associated protein 1	Tspoap1	13.3396
D3ZHB8	RCG23725, isoform CRA_a	Tmem198	15.9873
P0C1S9	Sn1-specific diacylglycerol lipase beta	Daglb	15.3525
F1LQQ7	Sodium channel protein	Scn7a	14.2356
O88457	Sodium channel protein type 11 subunit alpha	Scn11a	16.1925
P21707	Synaptotagmin-1	Syt1	15.3999
Q08850	Syntaxin-4	Stx4	10.8729
D4A8X6	Transcription factor 7-like 2	Tcf7l2	16.3563
Q8VHX0	Voltage-dependent calcium channel gamma-3 subunit	Cacng3	15.6603
P54287	Voltage-dependent L-type calcium channel subunit beta-3	Cacnb3	16.0308
D4A3I0	Zinc finger RANBP2-type-containing 1	Zranb1	11.5503

Table 1 Differentially expressed proteins in the rat frontal cortex. (continued)

Accession No.	Protein name	Gene name	Intensity (log2)
Control group			
P35365	5-hydroxytryptamine receptor 5B	Htr5b	14.2484
G3V8W0	Adrenergic receptor, alpha 1d	Adra1d	12.5786
P22909	Alpha-2A adrenergic receptor	Adra2a	18.8543
D3ZHS8	APC membrane recruitment protein 3	Amer3	14.7728
P18090	Beta-1 adrenergic receptor	Adrb1	15.8183
Q62762	Casein kinase I isoform gamma-2	Csnk1g2	20.1727
Q2VUH7	Dixin	Dixdc1	14.7622
Q5XIX0	DnaJ homolog subfamily C member 14	Dnajc14	19.4396
D4ACM8	Frizzled class receptor 7	Fzd7	15.3105
F1LMU7	Gamma-aminobutyric acid receptor subunit alpha-2	Gabra2	14.7845
P28473	Gamma-aminobutyric acid receptor subunit gamma-3	Gabrg3	17.4933
F1LRA4	Glutamate receptor-interacting protein 1	Grip1	14.1056
Q9WTW1	Glutamate receptor-interacting protein 2	Grip2	14.2675
P42264	Glutamate receptor ionotropic, kainate 3	Grik3	18.3849
Q63273	Glutamate receptor ionotropic, kainate 5	Grik5	18.4289
Q9Z2X5	Homer protein homolog 3	Homer3	17.0218
P23385	Metabotropic glutamate receptor 1	Grm1	15.0059
P08911	Muscarinic acetylcholine receptor M5	Chrm5	20.5789
F1LM84	Nidogen-1	Nid1	16.1577
D4AAR2	Olfactory receptor	Olr241	14.3781
Q8K4I6	Protein chibby homolog 1	Cby1	13.6364
Q62768	Protein unc-13 homolog A	Unc13a	14.4589
Q2IBD1	Protein Wnt	Wnt2	17.5819
B1WBR9	Protein Wnt	Wnt5b	15.15
D4A7Z1	RIMS-binding protein 3	Rimbp3	18.4418
Q9WV48	SH3 and multiple ankyrin repeat domains protein 1	Shank1	13.1214
Q62968	Sodium channel protein type 10 subunit alpha	Scn10a	18.3424
P28572	Sodium- and chloride-dependent glycine transporter 1	Slc6a9	17.2464
Q63632	Solute carrier family 12 member 4	Slc12a4	17.6559
Q80WE1	Synaptic functional regulator FMR1	Fmr1	14.3822
P60881	Synaptosomal-associated protein 25	Snap25	13.4411
P97610	Synaptotagmin-12	Syt12	14.8695
Q925C0	Synaptotagmin-9	Syt9	16.4016
D3ZKC8	Vang-like protein	Vangl1	13.5963
Q8VHW9	Voltage-dependent calcium channel gamma-4 subunit	Cacng4	16.0029
Q9EQ60	Voltage-dependent T-type calcium channel subunit alpha-1H	Cacna1h	18.3305

In addition, six overlapping proteins including the activity- regulated cytoskeleton-associated protein, D(1B) dopamine receptor, metabotropic glutamate receptor 8, Otoferlin, TNF alpha-induced protein 3, and Eph receptor B2 were categorized into the cell signaling process. The decreased expression of those was observed in the depressive group relative to control (Figure 2A). The interaction of co-expressed proteins underlying the cell signaling process was identified by STITCH. Dexamethasone, a synthetic glucocorticoid agonist, may affect the frontal cortex function through the dopaminergic system (Figure 2B). Dexamethasone causes a change in corticosterone hormone resulting in dopamine deficits. The changes of dopamine leading to a direct malfunction of the D(1B) dopamine receptor and metabotropic glutamate receptor 8 as well as indirect effects on Eph receptor B2 and TNF alpha-induced protein 3. However, interaction results from STITCH have shown no detection of dexamethasone effect on activity- regulated cytoskeleton-associated protein, and otoferlin.

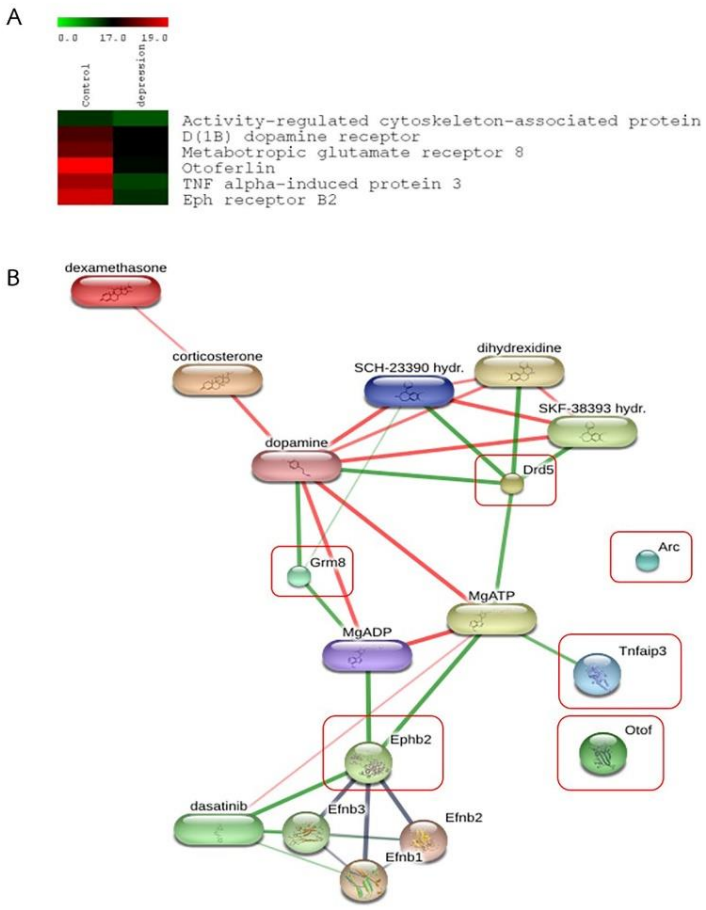

Figure 2 Analysis and characterization of overlapping protein during control and depressive group according to the cell signaling process. A) Heat-map representation of protein related to the cell signaling process (absent in green, low in dark green, and highest in red). B) Protein interaction mapping by STITCH show the correlation with the gene name of the identified protein in Heat- map (Activity- regulated cytoskeleton- associated protein (Arc), D(1B) dopamine receptor (Drd5), Metabotropic glutamate receptor 8 (Grm8), Otoferlin (Otof), TNF alpha-induced protein 3 (Tnfaip3), and Eph receptor B2 (Ephb2)).

Discussions and conclusions

The results of the study refer to the systematic identification and quantification of proteomic analysis in the protein profiles underlying the pathophysiology of depression. We found that proteome present differentially expressed protein including synaptotagmin, syntaxin, glutamate decarboxylase 2, 5- HT receptor, glutamate receptor, GABA receptor and activity- regulated cytoskeleton-associated protein according to mood status. The identified proteins in this study play an important role in the nervous system, particularly neurotransmitter receptors, enzymes, neurotransmitter transporters, voltage- dependent channels, and synaptosomal associated proteins. Consistent with previous studies, the reduction of the synaptosomal associated protein was found in several psychological diseases including bipolar disorder, schizophrenia, and depression [32]. The polymorphism of glutamic acid decarboxylase (GAD), the enzyme responsible for synthesizing GABA, was observed in anxiety disorders, major depression [33], and drug addiction [34]. This reveals that synaptic dysfunction plays an important role in the pathophysiology of neurological diseases, including schizophrenia and mood disorders. The results of this study also suggest that the neurotransmitter systems including the serotonin, norepinephrine, dopamine, glutamate, and GABA play an important role in the disease mechanisms. Previous studies have reported that the dysregulation of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) neurotransmission was found in a transgenic mouse model of depression, in which a downregulation of glucocorticoid receptors produced a dysfunction in the hypothalamic – pituitary – adrenal (HPA) axis [2]. An increased mRNA expression of alpha2A-adrenoceptors and serotonin receptors was observed in the post- mortem brains of depressed suicide victims [35]. The serotonin system is a target of selective serotonin reuptake inhibitors (SSRI), an important therapeutic drug of depression, that inhibit serotonin reabsorption resulting in elevation of the serotonin levels in the synaptic cleft [36]. This suggests that the impairment of serotonin system in both neurotransmitter levels and receptor protein expression leading to the development of the disease. Furthermore, the glutamatergic deficits show an important role in depression. N-methyl-D-aspartate (NMDA) receptor antagonists, 2- amino-7-phosphonoheptanoic acid [AP-7] and dizolcipine [MK-801], present antidepressant-like mechanisms in animal model of depression [9]. The reductions of metabotropic glutamate receptor 5 [37] and NMDA receptor subunits (NR2A and NR2B) [11] were found in depressed patients. Moreover, decreased GABA levels were observed in plasma, CSF and cortical cortex of depressed patients [13- 15]. $GABA_B$ receptor antagonists present the antidepressant- like effect in animal model of depression [12]. In addition, STITCH analysis revealed the abnormality of dopamine transmission underlying the influence of dexamethasone, a synthetic glucocorticoid receptor agonist, on mood status. Sucrose preference test was able to confirm that the depressive group exhibited the anhedonia symptom of depression relative to the control group (Kesyou et al., unpublished data). The dopamine neurotransmission system has also been found to play a role in depression through the reward circuit leading to anhedonia, sad mood and loss of pleasure [38]. The alteration of dopamine receptor expression was observed in learned helplessness model [39]. Interestingly, the present study shows the changes in other functioning proteins such as neuroligin-3, neuropeptide S, transcription factor 7-like 2, casein kinase I isoform gamma- 2, and

activity-regulated cytoskeleton-associated. These proteins have also been found to play a role underlying the pathophysiology of depression. Neuroligin-3 involves in the formation and remodeling of central nervous system synapses. Deletion of neuroligin-3 relate to autism spectrum disorders (ASDs), which may exhibit inhibitory synaptic transmission [40]. Altered expression of activity-regulated cytoskeleton-associated protein was found in glucocorticoid receptor-impaired (GR-i) mice a genetic model of depression [2]. This protein plays an important role in neuroplasticity. This evidence suggests that the identified proteins play an important role in the regulation of neurotransmission and the central nervous system responsible to mood status.

In conclusion, our finding confirms that the associated proteins in the cell signaling process play an important role in pathophysiology of depression, particularly synaptic transmission. These differentially expressed proteins may be potential biomarkers for depressive diagnostic and treatment. The expression of identified proteins should be further investigated with other methods such as western blot technique. Additionally, this observation might be a consequence of the dysfunction in the hypothalamic – pituitary –adrenal (HPA) axis, a chronic mild stress model induced by a synthetic glucocorticoid receptor agonist. Therefore, proteomic analysis should be confirmed in other animal models of depression and in depressed patients for clearly understanding of the disease mechanisms.

Acknowledgement

This study was financially supported by the agricultural research development agency (Public organization: PRP6205031230).

References

1. Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature. 2008;455 (7215):894-902.
2. Massart R, Mongeau R, Lanfumey L. Beyond the monoaminergic hypothesis: neuroplasticity and epigenetic changes in a transgenic mouse model of depression. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2012;367 (1601): 2485-94.
3. Luscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. Mol Psychiatry. 2011;16(4):383-406.
4. Moret C, Briley M. The importance of norepinephrine in depression. Neuropsychiatric disease and treatment. 2011;7(Suppl 1):9.
5. Anacker C, Zunszain PA, Carvalho LA, Pariante CM. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology. 2011;36(3):415-25.
6. Massart R, Mongeau R, Lanfumey L. Beyond the monoaminergic hypothesis: neuroplasticity and epigenetic changes in a transgenic mouse model of depression. Philosophical Transactions of the Royal Society B: Biological Sciences. 2012;367(1601):2485-94.
7. Placidi GP, Oquendo MA, Malone KM, Huang Y-Y, Ellis SP, Mann JJ. Aggressivity, suicide attempts, and depression: relationship to cerebrospinal fluid monoamine metabolite levels. Biological psychiatry. 2001;50(10):783-91.
8. Sarchiapone M, Carli V, Camardese G, Cuomo C, Di Giuda D, Calcagni M-L, et al. Dopamine transporter binding in depressed patients with anhedonia. Psychiatry Research: Neuroimaging. 2006;147(2-3):243-8.

9. Trullas R, Skolnick P. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. European journal of pharmacology. 1990;185(1):1-10.
10. Rajkowska G, Miguel-Hidalgo JJ, Dubey P, Stockmeier CA, Krishnan KRR. Prominent reduction in pyramidal neurons density in the orbitofrontal cortex of elderly depressed patients. Biological Psychiatry. 2005;58(4):297-306.
11. Feyissa AM, Chandran A, Stockmeier CA, Karolewicz B. Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2009;33(1):70-5.
12. Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, Cryan JF. Genetic and pharmacological evidence of a role for GABA B receptors in the modulation of anxiety- and antidepressant- like behavior. Neuropsychopharmacology. 2004;29(6):1050-62.
13. Petty F, Schlesser MA. Plasma GABA in affective illness: A preliminary investigation. Journal of affective disorders. 1981;3(4):339-43.
14. Gerner RH, Hare TA. CSF GABA in normal subjects and patients with depression, schizophrenia, mania, and anorexia nervosa. The American journal of psychiatry. 1981.
15. Honig A, Bartlett J, Bouras N, Bridges P. Amino acid levels in depression: a preliminary investigation. Journal of Psychiatric Research. 1988;22(3):159-64.
16. Sousa N, Cerqueira JJ, Almeida OF. Corticosteroid receptors and neuroplasticity. Brain research reviews. 2008;57(2):561-70.
17. Pariante CM. Risk factors for development of depression and psychosis: glucocorticoid receptors and pituitary implications for treatment with antidepressant and glucocorticoids. Annals of the New York Academy of Sciences. 2009;1179:144.
18. Sapolsky RM. The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. Biological psychiatry. 2000;48(8):755-65.
19. David DJ, Samuels BA, Rainer Q, Wang J-W, Marsteller D, Mendez I, et al. Neurogenesis-dependent and- independent effects of fluoxetine in an animal model of anxiety/ depression. Neuron. 2009;62(4):479-93.
20. Casarotto P, Andreatini R. Repeated paroxetine treatment reverses anhedonia induced in rats by chronic mild stress or dexamethasone. European Neuropsychopharmacology. 2007;17 (11):735-42.
21. Sigwalt A, Budde H, Helmich I, Glaser V, Ghisoni K, Lanza S, et al. Molecular aspects involved in swimming exercise training reducing anhedonia in a rat model of depression. Neuroscience. 2011;192:661-74.
22. Skupio U, Tertil M, Sikora M, Golda S, Wawrzczak- Bargiela A, Przewlocki R. Behavioral and molecular alterations in mice resulting from chronic treatment with dexamethasone: relevance to depression. Neuroscience. 2015;286:141-50.
23. Uys JD, Stein DJ, Daniels WM. Neuroproteomics: relevance to anxiety disorders. Current Psychiatry Reports. 2006;8(4):286-90.

24. Martins-de-Souza D, Harris LW, Guest PC, Turck CW, Bahn S. The role of proteomics in depression research. European archives of psychiatry and clinical neuroscience. 2010;260(6):499-506.
25. Bosch, P. J., Peng, L., & Kivell, B. M. Proteomics Analysis of Dorsal Striatum Reveals Changes in Synaptosomal Proteins following Methamphetamine Self- Administration in Rats. PloS one, 2015;10(10), e0139829.
26. Sharma A, Roytrakul S, Kittisenachai S, Semprasert N, and Kooptiwut S. 2020. Alteration in pancreatic protein expression in dexamethasone-treated mice. Songklanakarin J Sci Technol 42(3): 477-486.
27. Johansson C, Samskog J, Sundström L, Wadensten H, Björkesten L, Flensburg J. Differential expression analysis of Escherichia coli proteins using a novel software for relative quantitation of LC-MS/MS data. Proteomics. 2006;6(16):4475-85.
28. Thorsell A, Portelius E, Blennow K, Westman-Brinkmalm A. Evaluation of sample fractionation using micro-scale liquid-phase isoelectric focusing on mass spectrometric identification and quantitation of proteins in a SILAC experiment. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry. 2007;21(5):771-8.
29. Perkins DN, Pappin DJ, Creasy DM, Cottrell JS. Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis: An International Journal. 1999;20(18):3551-67.
30. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, et al. PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic acids research. 2017;45(D1):D183-D9.
31. Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. STITCH 5: augmenting protein–chemical interaction networks with tissue and affinity data. Nucleic acids research. 2016;44(D1):D380-D4.
32. Fatemi SH, Earle JA, Stary JM, Lee S, Sedgewick J. Altered levels of the synaptosomal associated protein SNAP-25 in hippocampus of subjects with mood disorders and schizophrenia. Neuroreport. 2001;12(15): 3257-62.
33. Hettema J, An S, Neale M, Bukszar J, Van den Oord E, Kendler K, et al. Association between glutamic acid decarboxylase genes and anxiety disorders, major depression, and neuroticism. Mol Psychiatry. 2006;11(8):752-62.
34. Veerasakul S, Watiktinkorn P, Thanoi S, Reynolds GP, Nudmamud-Thanoi S. Association of polymorphisms in GAD1 and GAD2 genes with methamphetamine dependence. Pharmacogenomics. 2017;18(1): 17-22.
35. Escribá PV, Ozaita A, García-Sevilla JA. Increased mRNA expression of alpha2A-adrenoceptors, serotonin receptors and mu-opioid receptors in the brains of suicide victims. Neuropsychopharmacology. 2004;29 (8):1512-21.

36. Yohn CN, Gergues MM, Samuels BA. The role of 5-HT receptors in depression. Molecular brain. 2017;10(1):1-12.
37. Deschwanden A, Karolewicz B, Feyissa AM, Treyer V, Ametamey SM, Johayem A, et al. Reduced metabotropic glutamate receptor 5 density in major depression determined by [11C] ABP688 PET and postmortem study. American Journal of Psychiatry. 2011;168 (7):727-34.
38. Heshmati M, Russo SJ. Anhedonia and the brain reward circuitry in depression. Current behavioral neuroscience reports. 2015;2(3): 146-53.
39. Kram ML, Kramer GL, Ronan PJ, Steciuk M, Petty F. Dopamine receptors and learned helplessness in the rat: an autoradiographic study. Progress in neuro-psychopharmacology & biological psychiatry. 2002.
40. Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, et al. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. science. 2007;318(5847):71-6.