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Research Article

Establishment of A Depressive Drug Screening Cell Platform

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1. Abstract

Depression is a problem that cannot be ignored. About 3% of people worldwide suffer from depression, but only 1/2 of the patients will seek medical help, and only 1/16 to 1/40 of the patients will be properly treated. Many people suffer from depression but have not been diagnosed or treated correctly. This is the biggest difficulty in treating depression, and it is also the main reason for the increasing burden of depression on society. According to the World Health Organization (WHO), among the top 10 diseases that increase social costs, depression is ranked third in 2004, but it is expected to become the first in 2030, indicating that the problems caused by depression need urgent attention from the society. A few days ago, WHO predicted that the three major diseases will affect worldwide in 2020, among which depression is ranked second, behind cardiovascular disease. Therefore, it is necessary to develop a set of drug screening cell platform for depression, which can quickly screen the target drugs for subsequent animal experiments and human experiments. In this study, PC-12 cells and SH-SY5Y cells were treated with 0.2 mmol/L corticosterone to simulate the depression-like pathological state of neurons in the brain. Depression-related indicators included cell survival, the expression of nerve growth factors (NGF), and intracellular Ca^{2+} ion concentration. It can be seen from these results that the PC-12 cells and SH-SY5Y cells in the corticosterone-treated group were significantly lower in cell survival and mRNA expression of NGF than the desipramine hydrochloride (DIM)-treated group and the control group. The intracellular Ca^{2+} ion concentration was significant increase in the corticosterone-treated group than DIM-treated group and the control group. According to these results, we have successfully established a cell platform for anti-depressive drug screening. We also hope it will be applied to verify the efficacy of the anti-depressive drug targets and explore the relevant mechanisms that caused depression.

2. Keywords

Cell platform; Depression; Drug screen; Establishment

3. Introduction

According to the report of World Health Organization (WHO), depression is the main cause of disability worldwide. Adults, adolescents, and children can be affected by this disease. Clinically, this disease is a mood disorder that involves a persistent feeling of sadness, and loss of interest. However, it is different from the mood fluctuations that people regularly experience as a part of life. Depression is an ongoing problem, not a passing one. It consists of episodes during which the symptoms last for at least 2 weeks. This disease also can last for several weeks, months, or years [1-4]. At present, it still does not fully understand the causes of depression. There are many possible causes and/or various factors combine to trigger symptoms. Many factors are likely to include as genetic features, neurotransmitter levels in brain, environmental factors, psychological factors, social factors, and others as bipolar disorder [5-7].

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Depression is treatable and its managing symptoms usually involves three treatable components. First component: support treatment can range from discussing practical solutions and possible causes to educating family members. Second component: psychotherapy, also known as talking therapy, some options include one-to-one counseling and cognitive behavioral therapy. Third component: antidepressant drug treatments that can be applied to treat the moderate-to-severe depression [8, 9].

Antidepressant drugs were first developed in the 1950s. However, their use has become progressively more common in the last 20 years. Now, main five classes of antidepressant drugs are available such as (1) selective serotonin reuptake inhibitors (SSRIs): they mainly affect serotonin despite of the other neurotransmitters. SSRIs are the most commonly prescribed antidepressant drugs. They are effective in treating depression and have fewer side effects than the other antidepressant drugs. SSRIs can block the reuptake or absorption of serotonin in the brain. Brain cells were easier to receive and send messages that resulted in better and more stable moods [8, 9]; (2) monoamine oxidase inhibitors (MAOIs): It inhibits the action of monoamine oxidase for decrease breaking down neurotransmitters as serotonin. There will be more circulating serotonin. In theory, this leads to more stabilized moods and less anxiety. If SSRIs have not worked, MAOIs will be considered to use in the depressant patients. MAOIs are generally saved for cases where other antidepressants have not worked because MAOIs interact with several other medications and some foods [1, 2]; (3) tricyclic antidepressants (TCAs): According to the chemical structure, there are three rings in the chemical structure of these medications. TCAs are used to treat depression, fibromyalgia, anxiety, and chronic pain control [3, 10]; (4) atypical antidepressants as noradrenaline and specific serotoninergic antidepressants (NASSAs): These are used to treat anxiety disorders, some personality disorders, and depression [5-7]; (5) selective serotonin and norepinephrine reuptake inhibitors (SNRIs): They are used to treat major depression, mood disorders, and possibly but less commonly attention deficit hyperactivity disorder, obsessive-compulsive disorder, anxiety disorders, menopausal symptoms, fibromyalgia, and chronic neuropathic pain. SNRIs play a key role in stabilizing mood via raising the levels of serotonin and norepinephrine [11, 12]. Each class acts on a different neurotransmitter or combination of neurotransmitters for therapying depression [13].

In this study, we want to establish a depressive drug screening cell platform (DDSCP) to quickly screen out targets for depression therapy and in the future, this DDSCP can be used to provide basic research on depression drug and therapeutic strategy development.

4. Materials and Methods

4.1. Cell Lines and Culture

A rat pheochromocytoma cell line used was PC-12 (ATCC® CRL-1721™). A human neuroblastoma cell lines used was SH-SY5Y (ATCC® CRL-2266™). PC-12 was maintained in Dulbecco's-modified Eagle's medium (DMEM; GIBCO®); SH-SY5Y was maintained in DMEM/F12 medium (GIBCO®). All cell lines were maintained in their specific media supplemented with 10% fetal bovine serum (FBS; HyClone®), 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen®), in a humidified 5% CO_2 incubator at 37°C.

4.2. Drugs and Reagents

Corticosterone (Cat No.: C2505) and desipramine hydrochloride (DIM; Cat No.: D3900) were ordered from Sigma-Aldrich Co. DMEM (Cat No.: 11885-084) and DMEM/F-12 (1:1) (Cat No.: 11320-033) were from GIBCO Co. Phosphate-buffered saline (PBS; Cat No.: 31717009) was ordered from Corning Co. Fetal bovine serum (Cat No.: AWE13438) was ordered from HyClone Co. Penicillin-Streptomycin (100×) (Cat No.: 15140-122), L-glutamine (Cat No.: 25030-81), TrypLE™ Express (Cat No.: 12605-010) were ordered from Invitrogen Co. Cell proliferation kit I (MTT) (Cat No.: 11465007001) was ordered from MERCK Co. Tissue total RNA mini kit (Cat No.: RT100) was ordered from Geneaid Co. Fura-2 AM (Ca^{2+} selective fluorescent indicator; Cat No.: ab120873) was ordered from Abcam Co.

4.3. Measurement of Cell Viability in PC-12 and SH-SY5Y Cells

Cell viability was measured by MTT [3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay according to the manufacturer's instructions (MERCK, Darmstadt, Germany). Finally, measurement was performed at 570 nm wavelength.

4.4. mRNA Expression of Nerve Growth Factors in PC-12 and SH-SY5Y Cells

Cells in each group were cultured in the 75T plastic flask (Costar) at a density of 2×10^8/L in the growth medium for 3-4 d. Then, the cells in each group were harvested and the total RNA was extracted by using total RNA mini kit, following the instructions provided by the manufacture. The yield and purity of total RNA was determined with Nanodrop spectrophotometer (ND-100; Thermo Fisher Scientific, DE, USA) at 260 and 280 nm.

RT-PCR was performed following the instruction provided by the manufacturer. One µg total RNA was added into 50 µL of RT-PCR reaction system containing 0.5 mmol/L Mg^{2+} ion concentration and a pair of primers (0.3 µg × 2). Sequences of the nerve growth factors (NGF) primers were followed as TCA TCC ACC CAC CCA

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GTC T (5') and CAC GCA GGC TGT ATC TAT C (3'). The expected size of NGF was 330 bp. The reaction mixtures were incubated for 45 min at 48℃ followed by 2 min at 94℃ to denature the template, and then thirty cycles (30 sec at 94℃, 1 min at 55℃, and 2 min at 72℃) and a final cycle (7 min at 72℃) were performed. The RT-PCR products were stored at 4℃. Later, ten μL RT-PCR products and λDNA (*EcoR* I/*Hind* III restriction digest) marker were subjected to electrophoresis in 2% agarose gel (GIBCO®)-containing 0.5 mg/L ethidium bromide (Cat No.: E1510; Sigma-Aldrich) and photographed under ultraviolet light.

4.5. Observation of Cell Morphology and Expression of Intracellular Ca^{2+} Ion Concentration in PC-12 and SH-SY5Y Cells

Cells were treated with/without one concentrations of corticosterone (0.2 mmol/L) and cultured for 24 h in 10% serum-containing culture medium. Observation of cell morphology with/without corticosterone treatment was applied with light microscope (Axiovert 40 CFL, Zeiss). Then, the intracellular Ca^{2+} ion concentration ($[Ca^{2+}]_i$) in PC-12 and SH-SY5Y cells under different treatments was detected by using the Ca^{2+}-sensitive dye Fura-2 AM according to the manufacturer.

4.6. Statistical Analysis

The results were expressed as mean ± SD. All statistical comparisons were made with two-tailed tests. Statistical evaluation was performed using SPSS 10.0 (SPSS Institute). Differences between groups were considered statistically significant at $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***, \%\%\%, \$\$\$}p < 0.001$.

5. Results

5.1. Measurement of Cell Viability in PC-12 and SH-SY5Y Cells

After the respective treatment of PC-12 and SH-SY5Y cells with 0.2 mmol/L corticosterone for 48 h, the OD_{570} values were significant decrease compared with control group ($p < 0.001$ in PC-12; $p < 0.05$ in SH-SY5Y), indicating that the cells were injured or dead. While in the presence of DIM (5 and10 μmol/L), the OD_{570} values were significantly reversed in a concentration-dependent manner, indicating that DIM could protect the cells from the corticosterone-induced cell injury (Figure 1).

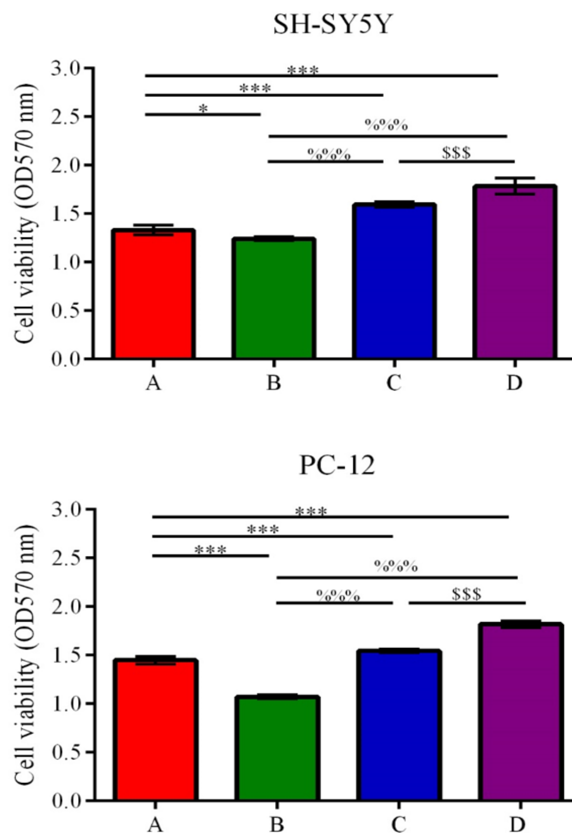

Figure 1: Effect of DIM on the 0.2 mmol/L corticosterone-decreased cell viability in PC-12 and SH-SY5Y cells. A: control group; B: 0.2 mmol/L corticosterone-treated group; C: 0.2 mmol/L corticosterone + 5 μmol/L DIM-treated group; D: 0.2 mmol/L corticosterone + 10 μmol/L DIM-treated group. Data were presented as Mean ± SD. $^{***, \%\%\%, \$\$\$}p < 0.001$

5.2. mRNA Expression of NGF in PC-12 and SH-SY5Y Cells

mRNA level of NGF in PC-12 and SH-SY5Y cells were determined by RT-PCR. Five and ten μmol/L DIM significantly increased the NGF mRNA level compared with the normal control in PC-12 and SH-SY5Y cells, indicating that the neuroprotection of anti-depressive drug, DIM might be associated with the increase in the expression of NGF (Figure 2). Additionally, 0.2 mmol/L corticosterone also be demonstrated that it can reduce the expression of NGF mRNA. After 5 and 10 μmol/ L DIM treatment, DIM can significantly revert the effect of the corticosterone-induced NGF mRNA expression (Figure 3).

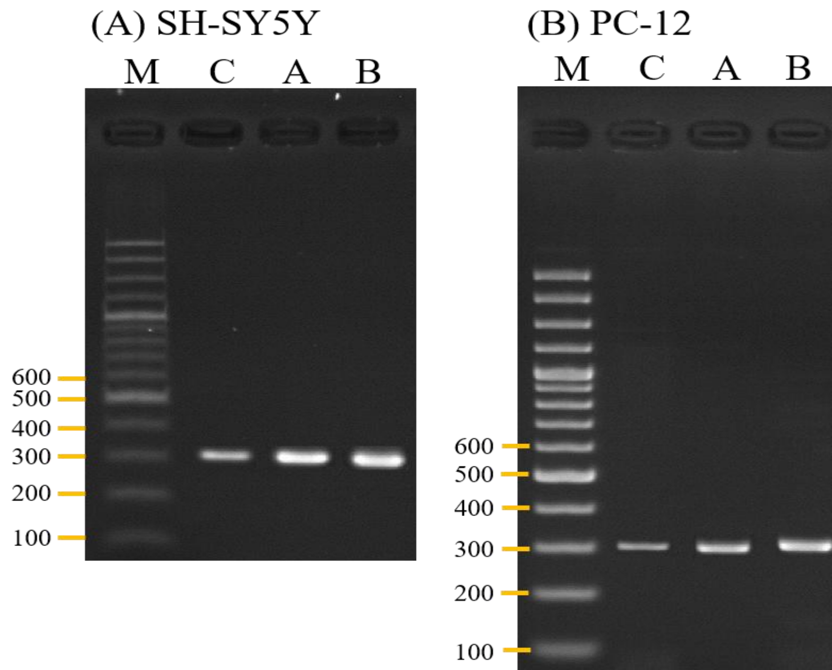

Figure 2: Effect of DIM on the NGF mRNA expression in PC-12 and SH-SY5Y cells. (A) Effect of DIM on the NGF mRNA expression in SH-SY5Y cells; (B) Effect of DIM on the NGF mRNA expression in PC-12 cells. M: marker; C: control group; A: 5 μmol/L DIM-treated group; B: 10 μmol/L DIM-treated group

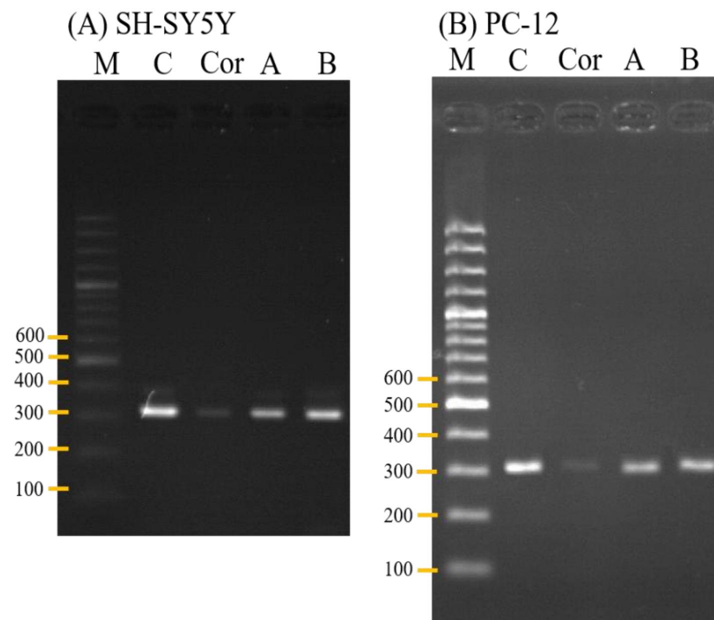

Figure 3: Effect of DIM on the 0.2 mmol/L corticosterone-reduced NGF mRNA expression in PC-12 and SH-SY5Y cells. (A) Effect of DIM on the corticosterone-reduced NGF mRNA expression in SH-SY5Y cells; (B) Effect of DIM on the corticosterone-reduced NGF mRNA expression in PC-12 cells. M: marker; C: control group; Cor: 0.2 mmol/L corticosterone-treated group; A: 0.2 mmol/L corticosterone + 5 μmol/L DIM-treated group; B: 0.2 mmol/L corticosterone + 10 μmol/L DIM-treated group

5.3. Observation of Cell Morphology and Expression of Intracellular Ca^{2+} Ion Concentration in PC-12 and SH-SY5Y Cells

After 0.2 mmol/L corticosterone treatment on PC-12 and SH-SY5Y cells for 48 h, intracellular $[Ca^{2+}]_i$ elevated significantly compared with the control. While in the presence of 5 and 10 µmol/L DIM, the corticosterone-induced $[Ca^{2+}]_i$ overloading was attenuated (Figure 4). These results indicated that the cytoprotective action of anti-depressive drug, DIM might be associated with its reducing the $[Ca^{2+}]_i$ overloading.

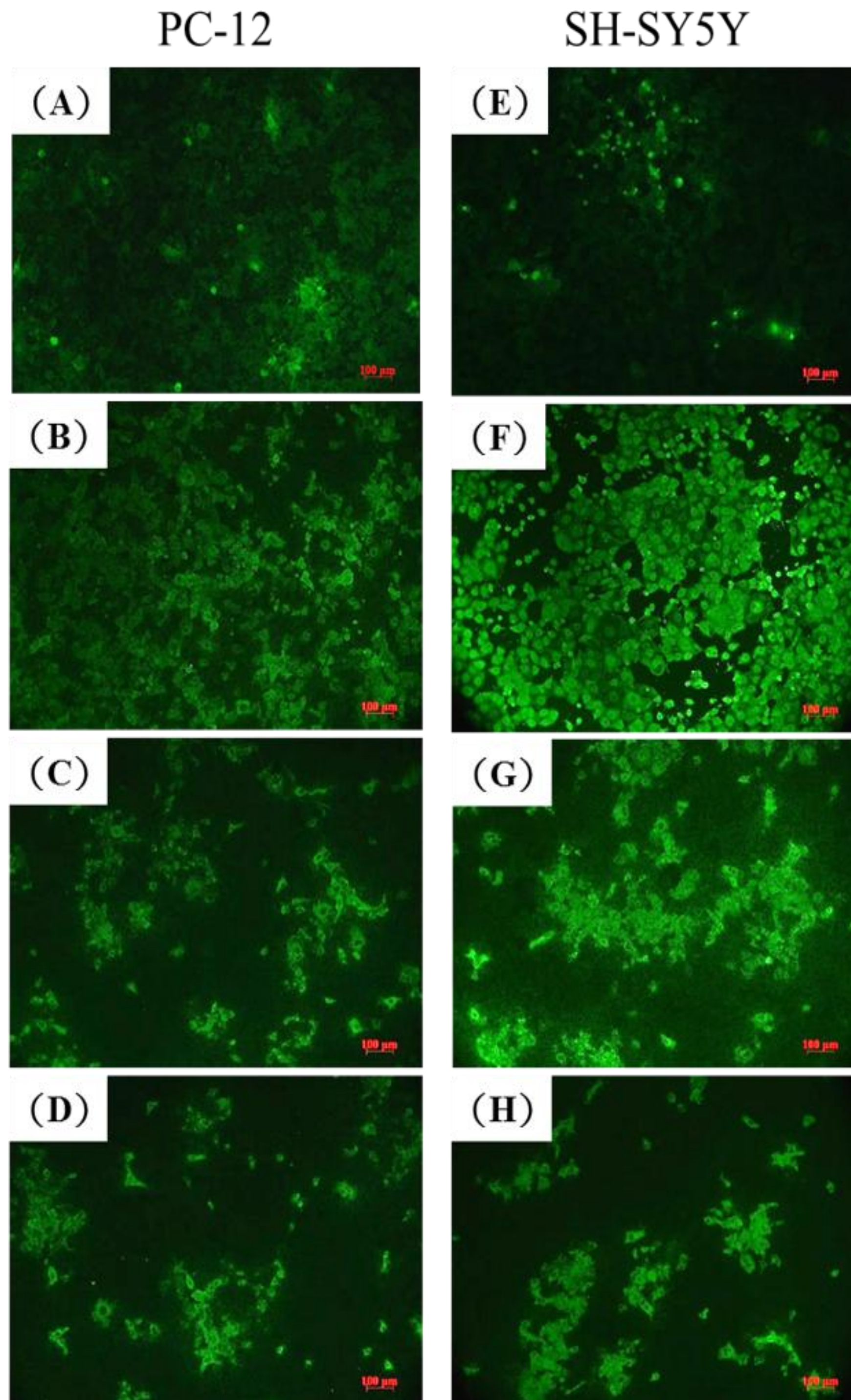

Figure 4: Effect of DIM on the 0.2 mmol/L corticosterone-induced $[Ca^{2+}]_i$ overloading in PC-12 and SH-SY5Y cells. (A-D) Effect of DIM on the corticosterone-induced $[Ca^{2+}]_i$ overloading in PC-12 cells; (E-H) Effect of DIM on the corticosterone-induced $[Ca^{2+}]_i$ overloading in SH-SY5Y cells;. (A and E) Control group; (B and F) 0.2 mmol/L corticosterone-treated group; (C and G) 0.2 mmol/L corticosterone + 5 µmol/L DIM-treated group; (D and H) 0.2 mmol/L corticosterone + 10 µmol/L DIM-treated group

6. Discussion

Depression is a complex/heterogeneous disorder which involved many factors in combination/alone may predispose person to the depressive risk. At present, *in vitro* and *in vivo* models of depression are established. Especially, many animal models of depression have already been established, which included the learned helplessness model [14], the unpredictable chronic mild stress model [4, 15, 16], the early life stress model [17], the olfactory bulbectomy model [18], the social defeat model [19], the chronic restraint stress model [20], the glucocorticoid/corticosterone model [21], the genetic models [22, 23], and the transgenic model [24]. These models each have varying degrees of face, construct, and predictive validity for depression and contribute differently to our understanding of antidepressant processes [25]. As animal welfare has gradually attracted attention in the recent years, the reduced animal pain and quantity, and increase of the experimental refinement are the important issues in the 3R of animal welfare. Although these animal experiments have good results for depressive research, however, the reduction of the experimental animal, the shortness of the experimental periods, low costs on the experiment, and low fault tolerance are now advocated. Therefore, *in vitro* depressive model for the rapid screening of drugs will be served as a testing efficacy platform for R&D of the anti-depressive drugs. The establishment of *in vitro* depressive model for the rapid drug screening and the elucidation of the related depressive mechanism is very need.

Many evidences were supported that the cytoprotective action might be the common pathway of the antidepressants [26-29]. Elevation of NGF expression also may be one of the mechanisms of cytoprotection of antidepressants, while the relationship between $[Ca^{2+}]_i$ overloading and NGF expression should be further studied. In this study, 0.2 mmol/L corticosterone caused cellular injury/damage and NGF decrease in PC-12 and SH-SY5Y cells to simulate an *in vitro* pattern of depression. After DIM administration, the cellular side effects of corticosterone can be reversed. Although the cellular side effects of corticosterone cannot be completely reversed, DIM can effectively reduce the deterioration of depression. Additionally, the change of $[Ca^{2+}]_i$ was also found in this *in vitro* model. Corticosterone increased $[Ca^{2+}]_i$, however, the effect of corticosterone on $[Ca^{2+}]_i$ can be reversed after DIM administration. According to these *in vitro* experimental data, the *in vitro* model of depression was successfully established.

7. Conclusion

Due to the relatively swift and specific models for new anti-depressive drug screening *in vitro* model are very few. We provide some evidences to speculate that the cytoprotective effect is one of the common action pathway for anti-depressive drug at present, which will contribute to the new anti-depressive drug screening and development. According to our data, the *in vitro* model of depression was successfully established. It is known that this successfully established cell platform for depressive drug screening and we hope that it will be applied to verify the efficacy of the depressive drug targets and explore the relevant mechanisms that caused depression.

References

1. Fasipe OJ. The emergence of new antidepressants for clinical use: Agomelatine paradox versus other novel agents. IBRO Rep. 2019; 6: 95-110.

2. Yeung AWK, Georgieva MG, Atanasov AG, Tzvetkov NT. Monoamine oxidases (MAOs) as privileged molecular targets in neuroscience: research literature analysis. Front Mol Neurosci. 2019; 12: 143.

3. Tamblyn R, Bates DW, Buckeridge DL, Dixon WG, Girard N, Haas JS et al. Multinational investigation of fracture risk with antidepressant use by class, drug, and indication. J Am Geriatr Soc. 2020; doi: 10.1111/jgs.16404.

4. Voorhees JL, Tarr AJ, Wohleb ES, Godbout JP, Mo X, Sheridan JF et al. Prolonged restraint stress increases IL-6, reduces IL-10, and causes persistent depressive-like behavior that is reversed by recombinant IL-10. PLoS One. 2013; 8: e58488.

5. Sie SD, Wennink JM, van Driel JJ, te Winkel AG, Boer K, Casteelen G et al. Maternal use of SSRIs, SNRIs and NaSSAs: practical recommendations during pregnancy and lactation. Arch. Dis. Child. Fetal Neonatal Ed. 2012; 97: F472-6.

6. Galling B, Calsina Ferrer A, Abi Zeid Daou M, Sangroula D, Hagi K, Correll CU. Safety and tolerability of antidepressant co-treatment in acute major depressive disorder: results from a systematic review and exploratory meta-analysis. Expert Opin Drug Saf. 2015; 14: 1587-608.

7. Wyska E. Pharmacokinetic considerations for current state-of-the-art antidepressants. Expert Opin Drug Metab Toxicol. 2019; 15: 831-47.

8. Eldar-Lissai A, Cohen JT, Meltzer-Brody S, Gerbasi ME, Chertavian E, Hodgkins P et al. Cost-effectiveness of brexanolone versus selective serotonin reuptake inhibitors for the treatment of postpartum depression in the United States. J Manag Care Spec Pharm. 2020; 19: 1-13.

9. Otto-Meyer S, DeFaccio R, Dussold C, Ladomersky E, Zhai L, Lauing KL, Bollu LR et al. A retrospective survival analysis of Glioblastoma patients treated with selective serotonin reuptake inhibitors. Brain Behav Immun Health. 2020; 2: 100025.

10. Varastehmoradi B, Wegener G, Sanchez C, Smith KL. Opioid system modulation of cognitive affective bias: implications for the treatment of mood disorders. Behav Pharmacol. 2020; 31: 122-35.

11. Castellano S, Torrent C, Petralia MC, Godos J, Cantarella RA, Ventimiglia A et al. Clinical and neurocognitive predictors of functional outcome in depressed patients with partial response to treatment: one year follow-up study. Neuropsychiatr Dis Treat. 2020; 16: 589-95.

12. Vulser H, Lemogne C, Boutouyrie P, Côté F, Perier MC, Van Sloten T et al. Depression, antidepressants and low hemoglobin level in the Paris Prospective Study III: A cross-sectional analysis. Prev Med. 2020; 7: 106050.

13. Sahli ZT, Banerjee P, Tarazi FI. The preclinical and clinical effects of vilazodone for the treatment of major depressive disorder. Expert Opin Drug Discov. 2016; 11: 515-23.

14. Higuchi F, Uchida S, Yamagata H, Abe-Higuchi N, Hobara T, Hara K et al. Hippocampal MicroRNA-124 enhances chronic stress resilience in mice. J Neurosci. 2016; 36: 7253-67.

15. Quesseveur G, Portal B, Basile J-A, Ezan P, Mathou A, Halley H et al. Attenuated levels of hippocampal connexin 43 and its phosphorylation correlate with antidepressant-and anxiolytic-like activities in mice. Front Cell Neurosci. 2015; 9: 490.

16. Ding H, Cui XY, Cui SY, Ye H, Hu X, Zhao HL et al. Depression-like behaviors induced by chronic corticosterone exposure via drinking water: Time-course analysis. Neurosci Lett. 2018; 687: 202-06.

17. Peña CJ, Neugut YD, Champagne FA. Developmental timing of the effects of maternal care on gene expression and epigenetic regulation of hormone receptor levels in female rats. Endocrinology. 2013; 154: 4340-51.

18. Hellweg R, Zueger M, Fink K, Hörtnagl H, Gass P. Olfactory bulbectomy in mice leads to increased BDNF levels and decreased serotonin turnover in depression-related brain areas. Neurobiol Dis. 2007; 25: 1-7.

19. Golden SA, Christoffel DJ, Heshmati M, Hodes GE, Magida J, Davis K et al. Epigenetic regulation of RAC1 induces synaptic remodeling in stress disorders and depression. Nat Med. 2013; 19: 337-44.

20. Zhang L, Luo J, Zhang M, Yao W, Ma X, Yu SY. Effects of curcumin on chronic, unpredictable, mild, stress-induced depressive-like be- haviour and structural plasticity in the lateral amygdala of rats. Int J Neuropsychopharmacol. 2014; 17: 793-806.

21. Dwivedi Y, Roy B, Lugli G, Rizavi H, Zhang H, Smalheiser N. Chronic corticosterone-mediated dysregulation of microRNA network in prefrontal cortex of rats: relevance to depression pathophysiology. Transl Psychiatry. 2015; 5: e682.

22. Nam H, Clinton SM, Jackson NL, Kerman IA. Learned helplessness and social avoidance in the Wistar-Kyoto rat. Front Behav Neurosci. 2014; 8: 109-27.

23. Willner P, Belzung C. Treatment-resistant depression: are animal models of depression fit for purpose?. Psychopharmacology. 2015; 232: 3473-95.

24. Gutknecht L, Popp S, Waider J, Sommerlandt FM, Göppner C, Post A et al. Interaction of brain 5-HT synthesis deficiency, chronic stress and sex differentially impact emotional behavior in Tph2 knockout mice. Psychopharmacology (Berl). 2015; 232: 2429-41.

25. Wang Q, Timberlake, II MA, Prall K, Dwivedi Y. The recent progress in animal models of depression. Prog Neuropsychopharmacol Biol Psychiatry. 2017; 77: 99-109.

26. Li YF, Liu YQ, Huang WC, Luo ZP. Cytoprotective effect is one of common action pathways for antidepressants. Acta Pharmacol Sin. 2003; 24: 996-1000.

27. Li YF, Luo ZP. Protection of buspirone on the cultured PC12 cells lesioned by corticosterone. Chin J Pharmacol Toxicol. 2001; 15: 333-6.

28. Li YF, Yang M, Yuan L, Zhao YM, Luan XH, Luo ZP. Antidepressant effect of quercetin 3-O-apiosyl (1→2)-[rhamnosyl(1→6)]-glucoside in mice. Chin J Pharmacol Toxicol. 2000a; 14: 125-7.

29. Li YF, Yang M, Zhao YM, Luo ZP. Protective effect of the oligosaccharides extracted from Morinda officinalis on the PC12 cells lesioned by corticosterone. China J Chin Mater Med. 2000b; 25: 551-5.