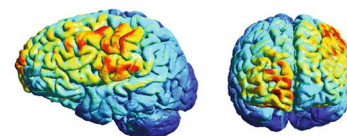


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The changes of tPA/PAI-1 system are associated with the ratio of BDNF/proBDNF in major depressive disorder and SSRIs antidepressant treatment

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25 **Conflicts of interest**

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32

Abstract Increasing evidence demonstrates that brain-derived neurotrophic factor (BDNF) can be regarded as a biomarker for major depression. Our previous work found that the ratio of mature BDNF (mBDNF) to precursor-BDNF (proBDNF) was a pivotal factor in the pathogenesis of major depressive disorder (MDD). But the mechanism behind the ratio is still obscure. Tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) both play essential roles in depression by regulating the ratio of BDNF/proBDNF. In present study, we analyzed BDNF, proBDNF, tPA and PAI-1 in the peripheral blood in 57 MDD patients pre- and post-treatment and in 57 healthy controls. We verified that BDNF and tPA levels were significantly decreased, whereas proBDNF and PAI-1 levels elevated obviously in MDD group pre-treatment. And after 4 weeks SSRIs treatment, the BDNF and tPA levels increased while the proBDNF and PAI-1 levels reduced. The MDD pre-treatment group had the lowest ratio of BDNF to proBDNF compared to MDD post-treatment group and control group. Though the ratio of tPA/PAI-1 in MDD pre-treatment had not reached the significance, it was still the lowest one among the three groups. The combination of tPA+PAI+BDNF showed the best diagnostic value for MDD. In summary, our data suggested that the interaction between tPA and PAI-1 implicated to the MDD and the antidepressant treatment which might through regulating the BDNF/proBDNF ratio. The combination of tPA, PAI-1 and BDNF might offer a helpful way for MDD diagnosis.

Key words: major depressive disorder, brain-derived neurotrophic factor, tissue plasminogen activator, plasminogen activator inhibitor-1

Introduction

The main clinical manifestations of major depressive disorder (MDD) are depressed mood, lack of interest and anhedonia. It has always been a research hotspot due to its high prevalence, suicide rate, susceptibility to recurrence and disability. According to World Health Organization (WHO), over 300 million people worldwide suffer from depression (World Health Organization, 2017).

The pathophysiology of MDD is still uncertain. There are various hypotheses about it. Among them, the brain-derived neurotrophic factor (BDNF) hypothesis of depression

has received widespread attention. Accumulating evidence supported that BDNF played pivotal roles in the pathogenesis and prognosis of MDD. BDNF is synthesized as pre-pro-BDNF, which is cleaved to mature BDNF (mBDNF) and precursor of BDNF (proBDNF). ProBDNF and mBDNF exert opposite effects by binding to different receptors (Lu et al., 2005). ProBDNF mainly binds to p75 neurotrophic receptor (p75NTR), which facilitates negative effects on the morphology and function of neurons by reducing neural remodeling and synaptic plasticity. On the contrary, mBDNF binds to tropomyosin-related receptor kinase B (TrkB) with high-affinity mediating neuronal survival, growth, and plasticity (Lu et al., 2005; Woo, et al., 2005). Our previous study reported that in MDD patients, the level of proBDNF increased, while the mBDNF level and mBDNF/proBDNF ratio were downregulated when compared with the healthy controls in the serum and lymphocytes (Zhou et al., 2013). It is still a complex question why the balance of proBDNF/mBDNF is dysregulated in the pathological condition. One of the possibilities is that there is something wrong during the process of proBDNF cleaved into mBDNF, for instance, the inability or insufficiency to convert proBDNF into mBDNF. ProBDNF is proteolytically cleaved by proteases (plasmin) which activated by tissue plasminogen activator (tPA) extracellularly to produce mBDNF (Lee et al., 2001; Pang et al., 2004; Nagappan, et al., 2009). Plasminogen activator inhibitor-1 (PAI-1) can restrain the activity of tPA in the conversion of plasminogen into plasmin, also can regulate the cell survival and apoptosis (Schneider et al., 2008). One study has reported that the pathogenesis of depression and the mechanisms underlying the antidepressant therapeutic effect were related to the tPA/ BDNF pathway (Jiang et al., 2017).

By reviewing recent literatures, we hypothesized that disinhibition of tPA by reducing PAI-1 induced increase of mBDNF, which ameliorated the depressive symptoms, and those might be a possible pathway for SSRIs to exert antidepressive effects.

Material and methods

Participants

All the subjects were enrolled from the Mental Hospital of Yunnan Province from

January 2021 to June 2022. The study was approved by the Ethics Committee of the Mental Hospital of Yunnan Province and all protocols related to human experiments were conducted in accordance with the Declaration of Helsinki. The patients met the following criteria: (1) 18 to 60 years old; (2) meeting the diagnostic criteria for MDD according to the International Classification of Diseases 10 Version (ICD-10) criteria; (3) the score of Hamilton Rating Scale for Depression 24 items (HAMD-24) was more than 21 points. The exclusion criteria for patients: (1) previously diagnosed with bipolar disorder, schizophrenia, alcohol or drug abuse and dependence, organic mental disorders; (2) pregnant or lactating women; (3) with serious cardiac, pulmonary, hepatic or renal diseases or any type of tumor; (4) those who not understanding the purpose of the study or unwilling to sign the informed consent form. Before entering the study, all the patients had been drug-free for at least 4 weeks with any antidepressants. All the patients were treated with SSRIs, with the dosage of 40-60 mg/day for fluoxetine or 15-20mg/day for escitalopram during the whole hospitalization for at least 4 weeks. The peripheral blood was sampled and the assessments of HAMD-24 were conducted before (pre-treatment) and after 4 weeks of SSRIs treatment (post-treatment). Healthy controls were recruited without any mental diseases, positive family history of psychiatric disorders or severe physical diseases who underwent physical examination in the hospital during the same period, and whose scores of HAMD-24 were less than 8 points.

Blood collection

Following an overnight fast, peripheral blood samples were collected into EDTA tubes between 7 a.m. and 9 a.m. The blood samples were collected both pre-and post-treatment (after 4 weeks of SSRIs treatment). The detailed methods of isolation of lymphocytes and the protein extraction were described in our previous paper in the year of 2013 (Zhou et al., 2013).

ELISA assay of plasma proBDNF and BDNF

The blood samples were centrifuged at 1500 g at 4 °C for 20 min to separate the plasma and then stored at -80 °C until assay. The plasma protein concentrations of BDNF and

proBDNF were assessed through enzyme linked immunosorbent assay (ELISA) by ELISA kit (proBDNF: DY3175 and BDNF: DY397; R&D Systems) following the manufacturer's instructions. All the experiments were performed in technical duplicate. All the samples have completed the ELISA assay.

Western blotting

As we had interpreted in our previously paper (Zhou et al., 2013), after the isolation of lymphocytes, the protein was extracted from the lymphocytes. Then 30 µg lymphocytes protein was separated on sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels and transferred onto polyvinylidene fluoride (PVDF) membranes (400 mA, 2 h), then blocked with 5% skim milk in Tris-buffered saline containing 0.05% Tween 20 (TBST). The membranes were incubated with primary antibodies for PAI-1 (1:1000, cat.#Ab187263; Abcam), tPA(1:1000, cat.#Ab157469; Abcam) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000, cat.#10494-1-AP; Proteintech) overnight. After the membranes were washed, they were incubated with goat anti-rabbit (1:5000, cat.#ab97051; Abcam) at room temperature for 1 hour. After washed again in TBST, enhanced chemiluminescent (ECL) substrate (cat.#cw0049, Cwbiotech; China) was applied on the membrane before imaging using ChemiDoc™ XRS + System (Bio-Rad, USA). For quantifying the protein level in lymphocytes, GAPDH was blotted as loading control. The blots were analyzed using Image J software (NIH, USA). All the samples were completed the western blotting.

Quantitative real-time PCR

The total RNA was extracted from the lymphocytes using the Trizol reagent (cat.#15596026CN, Invitrogen, USA). Subsequently, cDNA synthesis was through the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Code: RR047A, TaKaRa Biotechnology, China) according to the manufacturer's instruction. Gene expression levels of *BDNF*, *tPA* and *PAI-1* were detected by running quantitative real-time PCR (qRT-PCR) in ABI 7300 (ABI Applied Biosystems, USA) using TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) (Code: RR820A, TaKaRa Biotechnology,

China). The gene expression of *GAPDH* was detected as internal control.

The following primers were used:

BDNF forward: 5'- GGCTTGACATCATTGGCTGAC-3'

reverse: 5'- TACTGAGCATCACCTGGAC-3'

tPA forward: 5'- AGCGAGCCAAGGTGTTTCAA -3'

reverse: 5'- CTTCCCAGCAAATCCTTCGGG -3'

PAI-1 forward: 5'- GCACCACAGACGCGATCTT-3'

reverse: 5'- ACCTCTGAAAAGTCCACTTGC-3'

GAPDH forward: 5'- GGACCTGACCTGCCGTCTAG -3'

reverse: 5'- GTAGCCCAGGATGCCCTTGA -3'

All the primers were self-designed. The PCR cycling conditions were: 95 °C for 30 seconds, followed by 40 cycles at 95 °C for 15 seconds and 60 °C for 30 seconds and 95 °C for 1 minute, then 55°C for 30 seconds and 95 °C for 30 seconds. Samples were processed in technical triplicates.

Because there were not sufficient lymphocytes for both the western blotting and the qRT-PCR, there were 33 samples completed qRT-PCR in each group.

Statistical analysis

SPSS 18.0 software was used to analyze the data. Data were examined for normality using the Kolmogorov-Smirnov test. Demographic data and clinical characteristics were analyzed using Chi-squared or *t* test as appropriate. The Mann-Whitney *U* test was used in the comparison between the control group and the MDD pre-treatment group, also between the control group and the MDD post-treatment group. The Wilcoxon test (paired samples) was used to compare the changes between MDD pre- and post-treatment groups. Spearman correlation analysis was also conducted between the levels of tPA, PAI-1 and HAMD-24 scores. The area under the receiver operating characteristic (ROC) curve was calculated through logistic regression to determine the best diagnostic value of all the factors we assayed for MDD. Significance was set at $P < 0.05$.

Results

Demographic data of participants

A total of 114 participants were recruited, including 57 healthy controls and 57 MDD patients. There were no significant differences of demographic data in terms of age ($t = 0.575$, $P = 0.566$), gender ($\chi^2 = 0.650$, $P = 0.420$), ethnic ($\chi^2 = 0.211$, $P = 0.66$) and marriage status ($\chi^2 = 2.544$, $P = 0.267$). The HAMD scores were decreased after 4 weeks SSRIs antidepressants treatment in the MDD group ($t = 24.763$, $P < 0.001$) (Table 1).

The mRNA level and the plasma concentration of BDNF were reduced in the MDD patients before treatment and elevated after 4 weeks antidepressant treatment.

The plasma BDNF concentration of the MDD pre-treatment group dropped to 494.11(388.21, 792.31) pg/mL, while it in the control group was 1174.92(881.15, 1312.01) pg/mL. It obviously raised to 1193.86(728.33, 1423.83) pg/mL in the post-treatment group. The difference between control group and the MDD pre-treatment group was prominent ($U = 427.000$, $P < 0.001$, Mann-Whitney U test; Fig. 1A). The plasma BDNF concentration of MDD post-treatment group notably elevated compared to the pre-treatment group ($Z = -6.400$, $P = < 0.001$, Wilcoxon test; Fig. 1A) and had no significant difference compared to control group ($U = 1560.000$, $P = 0.715$, Mann-Whitney U test; Fig. 1A).

The mRNA level of *BDNF* of the MDD pre-treatment group was decreased slightly compared to the control group ($U = 470.000$, $P = 0.339$, Mann-Whitney U test; Fig. 1B). After 4 weeks antidepressive treatment, the mRNA level of *BDNF* in the MDD post-treatment group increased prominently ($Z = -3.922$, $P < 0.001$, Wilcoxon test; Fig. 1B) and also surpassed it of the control group ($U = 327.000$, $P = 0.005$, Mann-Whitney U test; Fig. 1B).

The plasma concentration of proBDNF was elevated in the MDD pre-treatment group and down-regulated after 4 weeks antidepressive treatment.

The plasma proBDNF concentration of the MDD pre-treatment group elevated to 597.39(489.54, 808.28) pg/mL, while the control group was 461.43(370.66, 592.81) pg/mL. The proBDNF concentration after 4 weeks SSRIs treatment decreased to 479.98(360.66, 753.40) pg/mL in the MDD post-treatment group. The plasma level of proBDNF in the MDD pre-treatment group was obviously higher than control group ($U = 1036.000$, $P = 0.001$, Mann-Whitney U test; Fig. 2) and the post-treatment group ($Z = -6.471$, $P = <0.001$, Wilcoxon test; Fig. 2). There were no significant differences between the control group and the MDD post-treatment group ($U = 1517.000$, $P = 0.542$, Mann-Whitney U test; Fig. 2).

The ratio of BDNF/proBDNF in the plasma markedly decreased in the MDD pre-treatment group and reversed after 4 weeks antidepressive treatment.

The ratio of BDNF/proBDNF in the MDD pre-treatment group was the lowest one among the three groups (compared to control group: $U = 455.000$, $P < 0.001$, Mann-Whitney U test; compared to post-treatment group: $Z = -6.567$, $P < 0.001$, Wilcoxon test; Fig. 3). There were no significant differences between control group and post-treatment group ($U = 1407.000$, $P = 0.218$; Mann-Whitney U test; Fig. 3).

The protein and mRNA levels of tPA were down-regulated in the MDD pre-treatment group and restored after 4 weeks antidepressive treatment.

The protein level of tPA in the lymphocytes was significantly declined in the MDD pre-treatment group compared with control group ($U = 1076.500$, $P = 0.002$, Mann-Whitney U test; Fig. 4A). After 4 weeks antidepressant treatment, the tPA protein level in the MDD post-treatment group rose to almost the same level as the control group ($U = 1623.000$, $P = 0.993$, Mann-Whitney U test; Fig. 4A) and apparently surpassed the level of pre-treatment group ($Z = -3.826$, $P < 0.001$, Wilcoxon test; Fig. 4A).

The mRNA level of *tPA* displayed almost the same trend as the protein level, but not reach the statistical significance (Fig. 4B). The *tPA* mRNA level of the control group has not statistical differences whether compared to the MDD pre-treatment group ($U = 449.000$, $P = 0.221$, Mann-Whitney U test; Fig. 4B) or the post-treatment group ($U =$

530.000, $P = 0.852$, Mann-Whitney U test; Fig. 4B). And no statistical difference of tPA mRNA level was found between pre- and post- treatment in the MDD patients ($Z = -1.081$, $P = 0.280$, Wilcoxon test; Fig. 4B).

The relationship between the western blotting results of tPA and HAMD-24 scores in the MDD pre-treatment group was analyzed. There was a weak negative correlation between the western blot results of tPA and HAMD-24 scores (Fig. 6A, $n = 57$, $r = -0.280$, $P = 0.036$).

The protein and mRNA levels of PAI-1 were increased in the MDD pre-treatment and downregulated after 4 weeks antidepressive treatment.

The protein level of PAI-1 in the lymphocytes was significantly enhanced in the MDD pre-treatment group compared with control group ($U = 1101.000$, $P = 0.003$, Mann-Whitney U test; Fig. 5A). After 4 weeks antidepressants treatment, the PAI-1 protein level of post-treatment group had not significant difference in comparison to the control group ($U = 1475.000$, $P = 0.397$, Mann-Whitney U test; Fig. 5A) and was lower appreciably when compared to the pre-treatment group ($Z = -5.828$, $P < 0.001$, Wilcoxon test; Fig. 5A)

The mRNA level of *PAI-1* in the MDD pre-treatment group was markedly up-regulated compared to control group ($U = 353.000$, $P = 0.014$, Mann-Whitney U test; Fig. 5B) and the post-treatment of MDD group ($Z = -2.600$, $P = 0.009$, Wilcoxon test; Fig. 5B) and there was no significant difference between control group and MDD post-treatment group ($U = 534.000$, $P = 0.893$, Mann-Whitney U test; Fig. 5B).

We analyzed the relationship between the western blot results of PAI-1 and HAMD-24 scores in the MDD pre-treatment group and found no significant correlation between them (Fig. 6A, $n = 57$, $r = 0.240$, $P = 0.072$).

Although the $tPA/PAI-1$ ratio decreased in the MDD pre-treatment group, it was not statistically significant.

The comparisons of the ratio of $tPA/PAI-1$ among control group, MDD pre-treatment group and post-treatment group showed that there were no statistical significances

among them. The ratio of tPA/PAI-1 in the MDD pre-treatment group was slightly lower than it in the control group ($U = 1481.000$, $P = 0.416$, Mann-Whitney U test; Fig. 6B) or that in the post-treatment group ($Z = -1.959$, $P = 0.050$, Wilcoxon test; Fig. 6B). There were no significant differences between control group and post-treatment group on the ratio of tPA/PAI-1 ($U = 1359.000$, $P = 0.132$; Mann-Whitney U test; Fig. 6B).

The diagnostic values of the combination of tPA+PAI-1+BDNF

Based on the above results, the changes of the western blotting results of tPA and PAI-1, the plasma levels of proBDNF and BDNF, the ratio of BDNF/proBDNF and tPA/PAI-1, we further assayed the diagnostic value of all the factors for diagnosis of MDD. The binary logistic regression model was used to estimate the predicted probability of factors developing MDD. The factors of the western blot results of tPA ($P = 0.06$), PAI-1 ($P = 0.015$) and the plasma levels of BDNF ($P = 0.010$) were enrolled at last. And the combined index of the western blotting results of tPA, PAI-1 and the plasma levels of BDNF could be used as a diagnostic indicator for MDD. The diagnostic values of all the factors were evaluated by ROC curve analysis and showed in Table 2 and Fig. 7. The combination of the western blot results of tPA, PAI-1 and the plasma levels of BDNF manifested the best clinical diagnostic power with the accuracy of 71.9%, sensitivity 89.5% and specificity 82.5%.

Discussion

Papers have suggested BDNF as a biomarker for depression (Zhou et al., 2013, Maffioletti et al., 2014; Zhou et al., 2017; Polyakova et al., 2018; Lin et al., 2021). In this research, the results also verified the earlier one. Our results demonstrated that the plasma level of BDNF was downregulated while the plasma proBDNF was upregulated in the MDD pre-treatment group compared with control group. However, after 4 weeks SSRIs treatment, the levels of both BDNF and proBDNF reversed. Moreover, we reported the ratio of BDNF/proBDNF in the plasma significantly decreased in the MDD pre-treatment group and restored 4 weeks antidepressive treatment later. It was consistent with the studies in human and animals (Sartori et al., 2011; Cao et al., 2014;

Jiang et al., 2017). Many antipsychotic and antidepressants drugs have similar effects of increasing expression of mBDNF and correcting the imbalance in mBDNF/proBDNF (Idell et al., 2017; Zhao et al., 2017; Yang et al., 2020). Physical exercises provide the antidepressive effects through altering the BDNF level as well (Cotman et al., 2002; Alizadeh, 2024). The above-mentioned strengthened the proposal that BDNF could be used as a biomarker for major depressive disorder, meanwhile it indicated that the imbalance or the insufficient conversion of proBDNF to BDNF might had a role in major depression (Zhou et al., 2013).

ProBDNF is cleaved into mBDNF under the promotion of plasmin, so the expression of plasmin affects the conversion of proBDNF into mBDNF, which may involve in the pathogenesis and prognosis of MDD. Plasminogen is a precursor of plasmin, which needs to be activated into plasmin under the action of tPA. Recent decades, with the in-depth research on BDNF, more and more articles have reported the role of tPA/plasmin in depression (Jiang et al., 2017; Zhou et al., 2017). TPA converts the precursor proBDNF to mBDNF by activating plasmin in the brain and PAI-1 is the major inhibitor of tPA (Lee et al., 2001; Pang et al., 2004; Melchor et al., 2005; Lee et al., 2015).

TPA is widely expressed in the brain. The mice which were knocked out the *tPA* gene in the hippocampus led to depressive and anxiety like behaviors, and increased the expression of *tPA* in the hippocampus could alleviate depression and anxiety symptoms (Bahi et al., 2012). Our study compared the lymphocytes protein and mRNA levels of tPA between the drug free MDD group and control group. The tPA lymphocytes protein level of MDD pre-treatment group decreased significantly compared to the control group, and ascended after 4 weeks antidepressant treatment. The mRNA trend was similar with the protein, though not reaching the significance. And the tPA level was negatively correlated with HAMD-24 scores, which meant that tPA was contributed to the severity of MDD. Our results confirmed the previous findings on the changes of tPA level in human and animals (Jiang et al., 2017; Han et al., 2019). In the MDD pre-treatment group, it is precisely because of the insufficient level of tPA that the cleavage of proBDNF into mBDNF is reduced, which is involved in the pathogenesis of MDD. The high level of tPA in post-treatment patients may have promoted a higher rate of

cleavage of proBDNF, contributing to the lower levels of proBDNF, the higher levels of BDNF and the BDNF/proBDNF ratio observed in this study (Sartori et al., 2001). The tPA activity is regulated by PAI-1, which is also the critical tPA inhibitor in vivo (Salles et al., 2002; Melchor et al., 2005). PAI-1 restrains the activity of tPA and inhibits the formation of plasmin and plasmin-mediated proteolysis. PAI-1 is considered as a key determinant of depression. The elevated levels of PAI-1 in cerebrospinal fluid or plasma are associated with depressive animals and human (Couch et al., 2016; Idell et al., 2017; Girard et al., 2019). Our results found that both the lymphocytes protein level and the mRNA level of PAI-1 were increased in the MDD group when compared with control group, and restored after SSRIs treatments. The correlation between lymphocytes protein level of PAI-1 and HAMD-24 scores is not significant, there is only a positive trend of correlation. All above suggested that the high level of PAI-1 might inhibit the tPA/plasmin system, reduce the cleavage of proBDNF into mBDNF, which led to decreased neuronal plasticity, direct impairing synaptic plasticity in patients with depression, which contributing to the development of depression. Previous research only discussed that the tPA/plasmin system might cause the imbalance of proBDNF to BDNF, but did not think about the effects of PAI-1 played on the tPA. The PAI-1 could interplay with tPA as a binding partner to mediate various effects on synaptic plasticity (Melchor et al., 2005). PAI-1 is primarily located in astrocytes and has been suggested to be anti-apoptotic for neurons in the brain (Soeda et al., 2008). The upregulation of PAI-1 is related to enhanced cell survival and apoptosis resistance (Schneider et al., 2008). This suggests that both tPA and PAI-1 may be neurotoxic or neurotrophic depending on their relative concentrations. The tPA/PAI-1 ratio is proved to be a potential therapeutic target for many pathological conditions, such as cardiovascular diseases (Sillen et al., 2020; Morrow et al., 2021), fibrosis (Rabieian et al., 2018), cancer (Ismail et al., 2021; Kubala et al., 2019), inflammatory and infectious diseases (Goolaerts et al., 2011; Lim et al., 2011; Huang et al., 2022). Whereas we inferred that the ratio of tPA to PAI-1 also contributed to depression. Our study found that the ratio of tPA/PAI-1 was declined in the MDD patients and reversed after SSRIs antidepressant treatment though the results did not

reach the statistical significance. The relationship between tPA and PAI-1 was interactive and the changes in their proportion could break the balance of mBDNF/proBDNF as well (Dong et al., 2020). Collectively, the results enlightened that the ratio of tPA/PAI-1 had a vital role in MDD pathophysiology by affecting the cleavage process of proBDNF to mBDNF (Tang et al., 2014). However, the results were inconsistent. Chen et al. reported that PAI-1 serum levels was just slightly lower, though tPA serum levels significantly decreased in MDD group than it in healthy controls (Chen et al., 2017), and concluded that serum level of PAI-1 were independent of MDD but related to the metabolic syndrome. The results still need to be validated in large-scale samples.

Our results found that the mRNA levels of *tPA* not reaching statistical significance among the three groups. The MDD pre-treatment group had the lowest level. It was discrepant with the western blotting results. By reviewing the papers, we found one research gave the explanation. Han et al. assayed the changes of tPA and PAI-1 in rats exposed to chronic unpredictable mild stress (CUMS) or lipopolysaccharide (LPS) and patients with depression, finally found tPA level failed to appear a significant difference in patients. Then the author proposed that stress or LPS induced the expression of PAI-1 and decreased tPA activity, but not tPA expression itself (Han et al., 2019). That was why the *tPA* mRNA level changed slightly, while the protein level altered obviously.

Our previous study (Zhou et al., 2013) proposed the diagnostic value of the serum mBDNF levels for MDD, the influence of tPA/PAI-1 was not involved yet. In the present study, we used the combination of the western blotting results of tPA+PAI-1 and the Elisa results of BDNF levels to explore the diagnostic value for MDD. The results showed better sensitivity and specificity (AUC = 0.928, sensitivity = 89.5%, specificity = 82.5% and accuracy = 71.9%), which indicated the combination was a preferred index for MDD diagnosis, and threw light on the understanding of MDD. Furthermore, it also indicates that depression is a complex disease, its pathogenesis is not caused only by the change of a single neurotransmitter. It may be due to both genetic and environmental influences that lead to the changes in a cascade of factors, resulting in the onset of depression.

In clinical practice, it has also been found that the use of antidepressants alone usually does not lead to complete remission. There were many studies and meta-analysis reported the improvement of depressive symptoms through exercise, and some have even compared exercise with antidepressant medications, which suggesting that physical exercises can exert their antidepressant effects by altering the expression of BDNF (da et al., 2023; Jemni et al., 2023; Noetel et al., 2024). However, due to the heterogeneity of depression and different exercise prescription parameters (i.e., frequency, intensity, time, type) reported, further research is needed for repeated trials and clinical applications. In a word, exercise as an adjunctive treatment for depression can enhance response, meanwhile reduce recurrence after discontinuation of medication.

There were several limitations in our research. Firstly, in the present study, we only measured the expressions of tPA, PAI-1, proBDNF and BDNF, but didn't assay the factors would affect the levels of tPA and PAI-1. For example, the PAI-1 level is also modulated by glucocorticoids, aldosterone and angiotensin (Hoirisch-Clapauch et al., 2016) and other metabolic related factors. Secondly, we did not measure the proinflammatory cytokines levels in the plasma and lymphocytes, which would affect the expressions of PA, PAI-1, proBDNF and BDNF. **Thirdly, we did not fully consider the role of exercise in alleviating depressive symptoms and altering BDNF levels, which may have led to variability of a few data.** Future studies should consider the above limitations. Finally, the number of the sample recruited was small, which should be confirmed in larger cohorts.

In summary, the present study verified our previous paper that BDNF and proBDNF played opposite effects in MDD. The interactive acts of tPA and PAI-1 contributed to the MDD and the antidepressive treatment which might through regulating the BDNF/proBDNF ratio. It showed clearly that the combination of tPA, PAI and BDNF had better diagnostic value in clinical scanning for MDD.

Reference:

- Alizadeh Pahlavani H. (2024). Possible role of exercise therapy on depression: Effector neurotransmitters as key players. *Behavioural brain research*, 459, 114791. <https://doi.org/10.1016/j.bbr.2023.114791>
- Bahi, A., & Dreyer, J. L. (2012). Hippocampus-specific deletion of tissue plasminogen activator “tPA” in adult mice impairs depression- and anxiety-like behaviors. *European neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology*, 22(9), 672–682. <https://doi.org/10.1016/j.euroneuro.2012.01.008>
- Cao, W., Duan, J., Wang, X., Zhong, X., Hu, Z., Huang, F., Wang, H., Zhang, J., Li, F., Zhang, J., Luo, X., & Li, C. Q. (2014). Early enriched environment induces an increased conversion of proBDNF to BDNF in the adult rat's hippocampus. *Behavioural brain research*, 265, 76–83. <https://doi.org/10.1016/j.bbr.2014.02.022>
- Chen, S., Jiang, H., Liu, Y., Hou, Z., Yue, Y., Zhang, Y., Zhao, F., Xu, Z., Li, Y., Mou, X., Li, L., Wang, T., Zhao, J., Han, C., Sui, Y., Wang, M., Yang, Z., Lu, Y., Zhu, Y., Li, J., ... Yuan, Y. (2017). Combined serum levels of multiple proteins in tPA-BDNF pathway may aid the diagnosis of five mental disorders. *Scientific reports*, 7(1), 6871. <https://doi.org/10.1038/s41598-017-06832-6>
- Cotman, C. W., & Berchtold, N. C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends in neurosciences*, 25(6), 295–301. [https://doi.org/10.1016/s0166-2236\(02\)02143-4](https://doi.org/10.1016/s0166-2236(02)02143-4)
- Couch, Y., Trofimov, A., Markova, N., Nikolenko, V., Steinbusch, H. W., Chekhonin, V., Schroeter, C., Lesch, K. P., Anthony, D. C., & Strekalova, T. (2016). Low-dose lipopolysaccharide (LPS) inhibits aggressive and augments depressive behaviours in a chronic mild stress model in mice. *Journal of neuroinflammation*, 13(1), 108. <https://doi.org/10.1186/s12974-016-0572-0>
- da Cunha, L. L., Feter, N., Alt, R., & Rombaldi, A. J. (2023). Effects of exercise training on inflammatory, neurotrophic and immunological markers and neurotransmitters in people with depression: A systematic review and meta-analysis. *Journal of*

- 448 [affective disorders, 326, 73–82. https://doi.org/10.1016/j.jad.2023.01.086](https://doi.org/10.1016/j.jad.2023.01.086)
- 449 Dong, Y., Hong, W., Tang, Z., Gao, Y., Wu, X., & Liu, H. (2020). Sevoflurane leads
450 to learning and memory dysfunction via breaking the balance of tPA/PAI-1.
451 *Neurochemistry international*, 139, 104789.
452 <https://doi.org/10.1016/j.neuint.2020.104789>
- 453 Girard, R. A., Chauhan, P. S., Tucker, T. A., Allen, T., Kaur, J., Jeffers, A., Koenig, K.,
454 Florova, G., Komissarov, A. A., Gaidenko, T. A., Chamiso, M. B., Fowler, J.,
455 Morris, D. E., Sarva, K., Singh, K. P., Idell, S., & Idell, R. D. (2019). Increased
456 expression of plasminogen activator inhibitor-1 (PAI-1) is associated with
457 depression and depressive phenotype in C57Bl/6J mice. *Experimental brain*
458 *research*, 237(12), 3419–3430. <https://doi.org/10.1007/s00221-019-05682-0>
- 459 Goolaerts, A., Lafargue, M., Song, Y., Miyazawa, B., Arjomandi, M., Carlès, M., Roux,
460 J., Howard, M., Parks, D. A., Iles, K. E., & Pittet, J. F. (2011). PAI-1 is an essential
461 component of the pulmonary host response during *Pseudomonas aeruginosa*
462 pneumonia in mice. *Thorax*, 66(9), 788–796.
463 <https://doi.org/10.1136/thx.2010.155788>
- 464 Han, W., Dang, R., Xu, P., Li, G., Zhou, X., Chen, L., Guo, Y., Yang, M., Chen, D., &
465 Jiang, P. (2019). Altered fibrinolytic system in rat models of depression and
466 patients with first-episode depression. *Neurobiology of stress*, 11, 100188.
467 <https://doi.org/10.1016/j.ynstr.2019.100188>
- 468 Hoirisch-Clapauch, S., Amaral, O. B., Mezzasalma, M. A., Panizzutti, R., & Nardi, A.
469 E. (2016). Dysfunction in the coagulation system and schizophrenia. *Translational*
470 *psychiatry*, 6(1), e704. <https://doi.org/10.1038/tp.2015.204>
- 471 Huang, P., Zuo, Q., Li, Y., Oduro, P. K., Tan, F., Wang, Y., Liu, X., Li, J., Wang, Q.,
472 Guo, F., Li, Y., & Yang, L. (2022). A Vicious Cycle: In Severe and Critically Ill
473 COVID-19 Patients. *Frontiers in immunology*, 13, 930673.
474 <https://doi.org/10.3389/fimmu.2022.930673>
- 475 Idell, R. D., Florova, G., Komissarov, A. A., Shetty, S., Girard, R. B., & Idell, S. (2017).
476 The fibrinolytic system: A new target for treatment of depression with psychedelics.

- Medical hypotheses, 100, 46–53. <https://doi.org/10.1016/j.mehy.2017.01.013>
- Ismail, A. A., Shaker, B. T., & Bajou, K. (2021). The Plasminogen-Activator Plasmin System in Physiological and Pathophysiological Angiogenesis. *International journal of molecular sciences*, 23(1), 337. <https://doi.org/10.3390/ijms23010337>
- Jemni, M., Zaman, R., Carrick, F. R., Clarke, N. D., Marina, M., Bottoms, L., Matharoo, J. S., Ramsbottom, R., Hoffman, N., Groves, S. J., Gu, Y., & Konukman, F. (2023). Exercise improves depression through positive modulation of brain-derived neurotrophic factor (BDNF). A review based on 100 manuscripts over 20 years. *Frontiers in physiology*, 14, 1102526. <https://doi.org/10.3389/fphys.2023.1102526>
- Jiang, H., Chen, S., Li, C., Lu, N., Yue, Y., Yin, Y., Zhang, Y., Zhi, X., Zhang, D., & Yuan, Y. (2017). The serum protein levels of the tPA-BDNF pathway are implicated in depression and antidepressant treatment. *Translational psychiatry*, 7(4), e1079. <https://doi.org/10.1038/tp.2017.43>
- Kubala, M. H., & DeClerck, Y. A. (2019). The plasminogen activator inhibitor-1 paradox in cancer: a mechanistic understanding. *Cancer metastasis reviews*, 38(3), 483–492. <https://doi.org/10.1007/s10555-019-09806-4>
- Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science (New York, N.Y.)*, 294(5548), 1945–1948. <https://doi.org/10.1126/science.1065057>
- Lee, T. W., Tsang, V. W., & Birch, N. P. (2015). Physiological and pathological roles of tissue plasminogen activator and its inhibitor neuroserpin in the nervous system. *Frontiers in cellular neuroscience*, 9, 396. <https://doi.org/10.3389/fncel.2015.00396>
- Lim, J. H., Woo, C. H., & Li, J. D. (2011). Critical role of type 1 plasminogen activator inhibitor (PAI-1) in early host defense against nontypeable *Haemophilus influenzae* (NTHi) infection. *Biochemical and biophysical research communications*, 414(1), 67–72. <https://doi.org/10.1016/j.bbrc.2011.09.023>
- Lin, L., Fu, X. Y., Zhou, X. F., Liu, D., Bobrovskaya, L., & Zhou, L. (2021). Analysis of blood mature BDNF and proBDNF in mood disorders with specific ELISA

- assays. *Journal of psychiatric research*, 133, 166–173.
<https://doi.org/10.1016/j.jpsychires.2020.12.021>
- Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nature reviews. Neuroscience*, 6(8), 603–614. <https://doi.org/10.1038/nrn1726>
- Maffioletti, E., Zanardini, R., Gennarelli, M., & Bocchio-Chiavetto, L. (2014). Influence of clotting duration on brain-derived neurotrophic factor (BDNF) dosage in serum. *Bio Techniques*, 57(3), 111–114. <https://doi.org/10.2144/000114204>
- Melchor, J. P., & Strickland, S. (2005). Tissue plasminogen activator in central nervous system physiology and pathology. *Thrombosis and haemostasis*, 93(4), 655–660. <https://doi.org/10.1160/TH04-12-0838>
- Morrow, G. B., Whyte, C. S., & Mutch, N. J. (2021). A Serpin With a Finger in Many PAIs: PAI-1's Central Function in Thromboinflammation and Cardiovascular Disease. *Frontiers in cardiovascular medicine*, 8, 653655. <https://doi.org/10.3389/fcvm.2021.653655>
- Nagappan, G., Zaitsev, E., Senatorov, V. V., Jr, Yang, J., Hempstead, B. L., & Lu, B. (2009). Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. *Proceedings of the National Academy of Sciences of the United States of America*, 106(4), 1267–1272. <https://doi.org/10.1073/pnas.0807322106>
- Noetel, M., Sanders, T., Gallardo-Gómez, D., Taylor, P., Del Pozo Cruz, B., van den Hoek, D., Smith, J. J., Mahoney, J., Spathis, J., Moresi, M., Pagano, R., Pagano, L., Vasconcellos, R., Arnott, H., Varley, B., Parker, P., Biddle, S., & Lonsdale, C. (2024). Effect of exercise for depression: systematic review and network meta-analysis of randomised controlled trials. *BMJ (Clinical research ed.)*, 384, e075847. <https://doi.org/10.1136/bmj-2023-075847>
- Pang, P. T., Teng, H. K., Zaitsev, E., Woo, N. T., Sakata, K., Zhen, S., Teng, K. K., Yung, W. H., Hempstead, B. L., & Lu, B. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science (New York, N.Y.)*, 306(5695), 487–491. <https://doi.org/10.1126/science.1100135>

- 534 Polyakova, M., Stuke, K., Schuemberg, K., Mueller, K., Schoenknecht, P., & Schroeter,
535 M. L. (2015). BDNF as a biomarker for successful treatment of mood disorders: a
536 systematic & quantitative meta-analysis. *Journal of affective disorders*, 174, 432–
537 440. <https://doi.org/10.1016/j.jad.2014.11.044>
- 538 Rabieian, R., Boshtam, M., Zareei, M., Kouhpayeh, S., Masoudifar, A., & Mirzaei, H.
539 (2018). Plasminogen Activator Inhibitor Type-1 as a Regulator of Fibrosis. *Journal*
540 *of cellular biochemistry*, 119(1), 17–27. <https://doi.org/10.1002/jcb.26146>
- 541 Salles, F. J., & Strickland, S. (2002). Localization and regulation of the tissue
542 plasminogen activator-plasmin system in the hippocampus. *The Journal of*
543 *neuroscience: the official journal of the Society for Neuroscience*, 22(6), 2125–
544 2134. <https://doi.org/10.1523/JNEUROSCI.22-06-02125.2002>
- 545 Sartori, C. R., Vieira, A. S., Ferrari, E. M., Langone, F., Tongiorgi, E., & Parada, C. A.
546 (2011). The antidepressive effect of the physical exercise correlates with increased
547 levels of mature BDNF, and proBDNF proteolytic cleavage-related genes, p11 and
548 tPA. *Neuroscience*, 180, 9–18. <https://doi.org/10.1016/j.neuroscience.2011.02.055>
- 549 Schneider, D. J., Chen, Y., & Sobel, B. E. (2008). The effect of plasminogen activator
550 inhibitor type 1 on apoptosis. *Thrombosis and haemostasis*, 100(6), 1037–1040.
- 551 Sillen, M.; Declerck, P.J. Targeting PAI-1 in Cardiovascular Disease: Structural
552 Insights Into PAI-1 Functionality and Inhibition. *Front. Cardiovasc. Med.* 2020, 7,
553 622473.
- 554 Soeda, S., Koyanagi, S., Kuramoto, Y., Kimura, M., Oda, M., Kozako, T., Hayashida,
555 S., & Shimeno, H. (2008). Anti-apoptotic roles of plasminogen activator inhibitor-
556 1 as a neurotrophic factor in the central nervous system. *Thrombosis and*
557 *haemostasis*, 100(6), 1014–1020. <https://doi.org/10.1160/th08-04-0259>
- 558 Tang, M., Jiang, P., Li, H., Cai, H., Liu, Y., Gong, H., & Zhang, L. (2015).
559 Antidepressant-like effect of n-3 PUFAs in CUMS rats: role of tPA/PAI-1 system.
560 *Physiology & behavior*, 139, 210–215.
561 <https://doi.org/10.1016/j.physbeh.2014.11.054>
- 562 Teng, H. K., Teng, K. K., Lee, R., Wright, S., Tevar, S., Almeida, R. D., Kermani, P.,
563 Torkin, R., Chen, Z. Y., Lee, F. S., Kraemer, R. T., Nykjaer, A., & Hempstead, B.

- L. (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 25(22), 5455–5463. <https://doi.org/10.1523/JNEUROSCI.5123-04.2005>
- World Health Organization (2017). Depression and other common mental disorders: global health estimates. Licence: CC BY - NC - SA 3.0 IGO
- Woo, N. H., Teng, H. K., Siao, C. J., Chiaruttini, C., Pang, P. T., Milner, T. A., Hempstead, B. L., & Lu, B. (2005). Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nature neuroscience*, 8(8), 1069–1077. <https://doi.org/10.1038/nn1510>
- Yang, C. R., Zhang, X. Y., Liu, Y., Du, J. Y., Liang, R., Yu, M., Zhang, F. Q., Mu, X. F., Li, F., Zhou, L., Zhou, F. H., Meng, F. J., Wang, S., Ming, D., & Zhou, X. F. (2020). Antidepressant Drugs Correct the Imbalance Between proBDNF/p75NTR/Sortilin and Mature BDNF/TrkB in the Brain of Mice with Chronic Stress. *Neurotoxicity research*, 37(1), 171–182. <https://doi.org/10.1007/s12640-019-00101-2>
- Zhao, G., Zhang, C., Chen, J., Su, Y., Zhou, R., Wang, F., Xia, W., Huang, J., Wang, Z., Hu, Y., Cao, L., Guo, X., Yuan, C., Wang, Y., Yi, Z., Lu, W., Wu, Y., Wu, Z., Hong, W., Peng, D., ... Fang, Y. (2017). Ratio of mBDNF to proBDNF for Differential Diagnosis of Major Depressive Disorder and Bipolar Depression. *Molecular neurobiology*, 54(7), 5573–5582. <https://doi.org/10.1007/s12035-016-0098-6>
- Zhou, C., Zhong, J., Zou, B., Fang, L., Chen, J., Deng, X., Zhang, L., Zhao, X., Qu, Z., Lei, Y., & Lei, T. (2017). Meta-analyses of comparative efficacy of antidepressant medications on peripheral BDNF concentration in patients with depression. *PloS one*, 12(2), e0172270. <https://doi.org/10.1371/journal.pone.0172270>
- Zhou, L., Xiong, J., Lim, Y., Ruan, Y., Huang, C., Zhu, Y., Zhong, J. H., Xiao, Z., & Zhou, X. F. (2013). Upregulation of blood proBDNF and its receptors in major

592 depression. Journal of affective disorders, 150(3), 776–784.
593 <https://doi.org/10.1016/j.jad.2013.03.002>