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A novel aptamer agent showed antidepressant function via binding 5-hydroxytryptamine receptor to block re-uptake of 5-HT

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Abstract

Background & Objectives: Systematic evolution of ligands by exponential enrichment (SELEX) technology was widely used to screen the aptamers that bind the target protein safely and efficiently. Our study aimed to screen aptamers for anti-depression via binding to the 5-HTR to block 5-HT re-uptake. *Methods:* The prokaryotic expression plasmid was constructed and the recombinant 5-HT_{1A}R (mice) was expressed and purified. The ssDNA aptamer that bound 5-HT_{1A}R specifically was screened by SELEX (Enzyme-linked Oligonucleotide assay), and the binding sites and relative binding strength of ssDNA were detected. At the same time, ssDNA aptamer inhibitory protein uptake and against depression was verified in cellular level and mouse depression model. *Results:* The recombinant 5-HT_{1A}R protein was purified successfully. After 12 rounds of positive screening and 5 rounds of negative screening, four aptamers with high affinity and specificity were obtained and the same epitope was bounded by four aptamers using ELONA (Enzyme-linked Oligonucleotide assay). The uptake of 5-HT was influenced by aptamer 18 in vitro, and the improvement of depression state in mice after intravenous injection of aptamer 18 was proved by tail suspension experiment in mice.
Conclusions: Aptamer is expected to be a new type of antidepressant, which can be used in the treatment of depression.

Keywords: Depression, serotonin receptor, selective serotonin reuptake inhibitors, SELEX, aptamer

INTRODUCTION

Depression is a high incidence rate of neuropsychiatric disorders, which can be found in all age groups.[1] At present, the etiology of major depression and related depression is complex and not fully elucidated[2,3], the most extensive depression hypothesis is related to the level of extracellular neurotransmitters.[4-6] Therefore, some substances can increase the level of monoamine and may be beneficial to the treatment of depression.[7] Among monoamine reuptake inhibitors, selective serotonin reuptake inhibitors (SSRIs) have been listed as one of the most effective substances. The mechanism of SSRIs is to selectively inhibit the reuptake of 5-hydroxytryptamine (5-HT) by the presynaptic membrane of the central nervous system and increase the concentration of 5-HT in synaptic space to achieve the therapeutic purpose.[8] Elevated 5-HT concentrations activate various post-synaptic 5-HT receptors in multiple regions of the brain, which can reduce depression symptoms.[9] In addition, high extracellular concentrations of 5-HT trigger a negative feedback mechanism involving 5-HT_{1A} receptors, which regulate 5-HT levels in synaptic spaces. High concentrations of endogenous 5-HT may be sufficient to inhibit acetylcholine receptors.[10]

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However, antidepressant drugs can lead to a series of side effects, such as headache, nausea and elevated transaminase. Also these drugs can result in serious adverse reactions like suicide, serotonin syndrome, and sexual dysfunction.[11] It is reported that more than 80% of patients have at least one adverse reaction, with an average of four adverse reactions per patient, many of these adverse reactions cause significant problems to patients and even affect daily functions.[12] Therefore, it is necessary to regularly monitor biochemical indicators to find problems and improve them in time.

The aptamers screened by the systematic evolution of ligands by exponential enrichment (SELEX) technology have a wide range of targets, convenient preparation and good stability, which are widely used in biomedical research.[13] Aptamers can functionally alter cell biological behavior, such as cell migration, cell proliferation, cell differentiation, autophagy and anti-inflammatory effects.[14] Aptamers also show great potential and advantages in the early diagnosis and treatment of tumors. At present, aptamers targeting tumor-related proteins have been screened and widely used in the research of tumor diagnosis and treatment.[15,16] In addition, aptamers are also used in the detection and treatment of neurotransmitters[17], a novel functional nucleic acid aptamer to amyloid-β peptide 1-40 (Aβ1-40) was developed for the detection of Alzheimer disease.[18] In our previous research, we constructed and applicated of ssDNA aptamers against glycolipid antigen ManLAM of mycobacterium tuberculosis for TB diagnosis.[19] Because of the above shortcomings of the existing drugs and the prospect of their application in the field of medicine, we intended to clone and express 5-HT_{1A}R as the target. Then we used SELEX technology to screen aptamers that can specifically bind 5-HTR, and to block the reuptake of 5-HT for the anti-depressant effect.

METHODS

8-week-old specific-pathogen-free (SPF) C57BL/6J female mice (weight 18~20g) were purchased from Nanjing Qinglongshan animal experimental base (Animal Certificate No: SCXK(Yu) 2020-0005); Mouse skeletal muscle myocyte line C3H (JRDUN bio, China); Reverse Transcriptase (Fermentas, USA); Primer were synthesized by Shanghai Sangon Biotech (China); His tagging column, SDS-PAGE gel preparation kit (Solibao company, China); Predyeing protein Marker (Fisher, USA); Isopropyl-β-d-thiogalactoside (IPTG) (Sigma, USA); HRP coupled mouse anti-His tag monoclonal antibody (Beyotime, China).

Research design

The prokaryotic expression plasmid was constructed and the recombinant 5-HT1AR (mice) was expressed and purified. The ssDNA aptamer that specifically bound to 5-HT1AR was screened using SELEX, and the binding sites and relative binding strength were detected. Additionally, the cellular level and mouse depression model were used to verify the inhibitory protein uptake and effectiveness of the ssDNA aptamer against depression.

Cloning and construction of 5-HT_{1A}R gene

C57BL/6J mice were killed by decapitation. After muscle tissue homogenate was taken, mouse RNA was extracted by Trizol method and transformed into cDNA by reverse transcription kit, (https://www.uniprot.org/uniprot/Q64264). Primers were designed according to NCBI (NM_008308): F: 5'-CGGATCCGATATGTTCAGTCT TGGCCAG-3' (underlined as BamH I restriction site). R: 5'-CAAGCTTGGCGGCA GAACTTGCACTTG-3' (underlined as Hind III restriction site). The amplified DNA strand and plasmid pET28a were digested with restriction endonuclease, then connected with T4 ligase, and the connecting product was transformed into DH5α which was cultured in Kanamycin resistant LB solid medium to screen positive monoclonal colonies. The plasmid was extracted from the amplified bacteria and identified by enzyme digestion.

Expression, purification and identification of 5-HT_{1A}R protein

The pET28a/5-HT_{1A}R plasmid was transformed into BL21 *E.coli* strains, IPTG(0.8mol/L) was added into the bacterial solution at a ratio of 1:1000, and induced expression at 25 °C for 4h. After the culture, the bacteria were collected by centrifugation and lysed by high-pressure homogenizer, supernatant was separated from the broken bacteria by centrifugation (4 °C, 10 000×G) for 30 min, then protease inhibitor PMSF was added to it. The recombinant 5-HT_{1A}R protein was purified by Ni NAT, and identified by West blot and peptide fingerprint with mouse anti-His tag monoclonal antibody.

Screening of 5-HT_{1A}R protein aptamers

Aptamer library was designed and synthesised as follow[20]: 5' -GCGGAATTCTAATACGACTCACTATAGGGAACAGTCCCGAGCC-(N30)-GGGTCAATGCGTCATA-3' (88 nt, Where N represents any base of A, T, G and C). Primers were designed as: P1: 5' -GCGGAATTCTAATACGACTCACTATAGGGAACAGTCCGAGCC-3': P2: 5' -GCGGGATCCTATGACGCATTGACCC-3'. The concentration of purified 5-HT_{1A}R protein was adjusted to 10mg/L, 1mg/L, 0.1mg/L and 0.01mg/L with carbonate buffer (PH=9.6), and 50uL proteins of different concentrations were coated in polystyrene micropores. One concentration was used every 3 rounds, and a total of 12 rounds of forwarding screening were carried out. 5 rounds of reverse screening were added from round 6 to round 10. The relative binding force of the library in each round was compared.

Screening of monoclonal aptamers and detection of binding ability

The aptamer library with the highest relative binding capacity was amplified into double stranded DNA (dsDNA) by PCR, the dsDNA was digested with BamH I and Hind III and subcloned into pUC19. The recombinant plasmid was transformed into *E.coli* DH5α bacteria and cultured overnight. The monoclonal colonies were picked from the culture plate and the plasmids were extracted for double digestion identification, and then the aptamers were amplified by asymmetric PCR. Enzyme-linked Oligonucleotide assay (ELONA) was used to detect the relative binding force of aptamers. The screened aptamers with high relative binding force were sequenced, and the consistency of their recognition sites was verified by competition method: the same amount of biotin labeled aptamers and non-labeled aptamers were put into the micropores precoated with 5-HT_{1A}R. HRP conjugated streptavidin was added and incubated at 37°C for 30 min. After adding the tetramethylbenzidine (TMB) substrate and stop buffer (2mol/L H_2SO_4), the absorbance values at OD450 were determined using a microplate reader.

Detection of specificity of aptamers

The 5' end of the monoclonal aptamers with the highest relative binding force screened in above-mentioned aptamers were labeled with FAM. The aptamers were incubated with mouse skeletal muscle myoblastic C3H cell line in different concentrations (0mmol/L, 10mmol/L, 20mmol/L, 40mmol/L and 80mmol/L) for 45 min in darkness. After washing with PBS, the binding force was observed and compared by flow cytometry.

Detection of aptamer blockade 5-HT uptake

High Performance Liquid Chromatography (HPLC, Angilent 1260, USA) was used to detect the standard concentration of 500 ng/mL 5-HT, the 5-HT initial concentration of 200 ng/mL 5-HT was added to C3H cell culture and the final concentration after 12h incubation. In addition, another group of C3H cells were incubated with 200 ng/mL 5-HT and aptamer 10 nM for 12 h, the final concentration of 5-HT was detected. After 12h, the cells were centrifuged at 1000g for 15min to collect the supernatant fluid.

The therapeutic effect of aptamer was evaluated by mouse tail suspension test

The mouse tail suspension test box was divided into four groups. Control group: mice were suspended for 6 min, and the rest time of mice was observed within 4 min after suspension. Aptamer treatment group: after injecting aptamer through caudal vein, mice were hung the tail for 6 minutes, and observed the resting time within 4 minutes. Reagent control group: normal saline was injected into caudal vein. Drug treatment group: 20 mg/kg fluoxetine orally. The resting time of mice within 4 min was also observed.

Statistical analysis

SPSS 22.0 software was used for statistical analysis. The measurement data were expressed as mean ± SD. T-test was used for the comparison between the two groups, and analysis of variance was used for the comparison between the three groups. There difference was considered statistically significant when $P < 0.05$.

RESULTS

Construction and identification of expression plasmid pET28a/5-HT_{1A}R

RNA from C57BL/6J mouse muscle cells was extracted and reverse transcribed into cDNA as a template. The coding region of 5-HT_{1A}R mRNA was amplified with designed primers. According to DNA agarose electrophoresis, there was a band between 1 000 bp and 2 000 bp, which was consistent with 1 272 bp from the expected

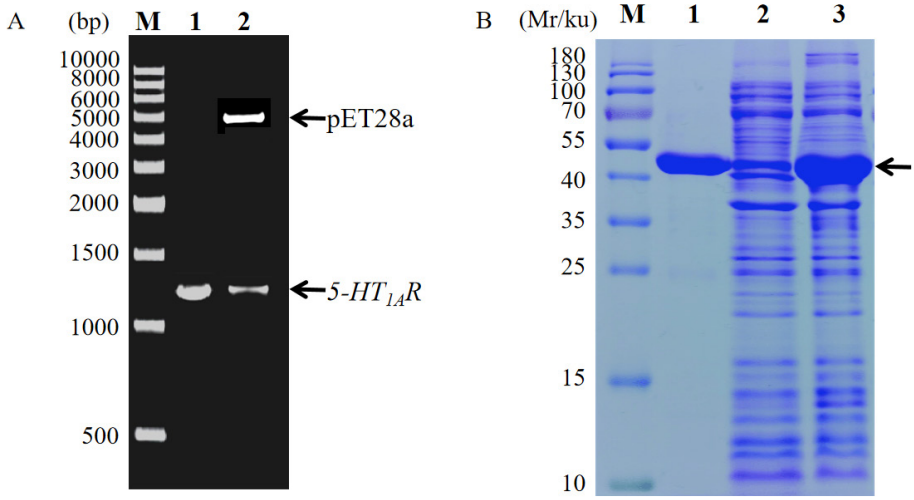

Figure 1. Identification of expression plasmid pET28a/5-HT$_{1A}$R and induced recombinant 5-HT$_{1A}$R by SDS-PAGE (A): M: DNA ladder; Lane 1:5-HT1AR gene of human was amplified by PCR; Lane 2: The constructed pET28a/5-HT1AR plasmid was identified by double enzyme digestion. Arrow: 5-HT1AR gene (about 1272 bp). (B): M: protein ladder; Lane 1: Purified recombinant human 5-HT$_{1A}$R protein; Lane2: Uninduced Protein of BL21 strain containing pET28a/5-HT$_{1A}$R recombinant plasmid without induce; Lane 3: Protein of BL21 strain containing pET28a/5-HT$_{1A}$R recombinant plasmid with IPTG-induce; Arrow: Recombinant 5-HT$_{1A}$R protein (molecular weight: 47kDa).

PCR amplification target band product (Figure 1). The constructed pET28a/5-HT$_{1A}$R plasmid was digested by double enzyme and analyzed by DNA agarose electrophoresis, it produced two bands, 5 300 bp and 1 270 bp respectively, which were consistent with the theory of the expected target product (Figure 1). The plasmid extracted from the positive monoclonal colony was sent to Shanghai Sangon for sequencing, and the results were consistent with Genebank (NM_000524), with no mutations, deletions or shifts.

5-HT$_{1A}$R expression and purification

The cloned pET28a/5-HT$_{1A}$R plasmid was transformed into *E.coli* BL21 (DE3), IPTG was used to induce its expression, and the protein was purified by Ni NAT affinity column. It can be seen that it has a significant expression band with a molecular weight of 40-55 kDa, which is consistent with the predicted molecular weight of 47 kDa (Figure 1). Through PMF identification, it is consistent with the 5-HT$_{1A}$R peptide sequence (Table 1).

Aptamer screening and characteristic identification

After 12 rounds of forwarding screening and 5 rounds of reverse screening, the 12th round of aptamer library with the highest binding ability was cloned into pUC19 plasmid and introduced into *E.coli* DH5α (Figure 2). A total of 51 positive clones were selected, of which clone were numbered and the aptamer 18, aptamer 25, aptamer 31 and aptamer 45 showed high binding ability respectively (Figure 3, Figure 4 and Figure 5). At the same time, we analyzed the specificity of the above four ssDNA base

Table 1: Analysis of the recombinant 5-HT1AR by peptide mass fingerprinting

No.	Amino acid sequence of peptide (N→C)	AA site	Likelihood Ratio
1	ACVVAAIALERSLQNVANYLIGSL	55~79	100%
2	VLCCTSSILHLCAIALDRYW	117~137	100%
3	GTPCANGAVRQGEDDA	272~288	100%
4	WLIGFLISIPPMLGWRT	161~178	100%
5	ALVLPFCESSCHMPELL	365~382	100%
6	YFNKDFQNAFKK	402~414	98%

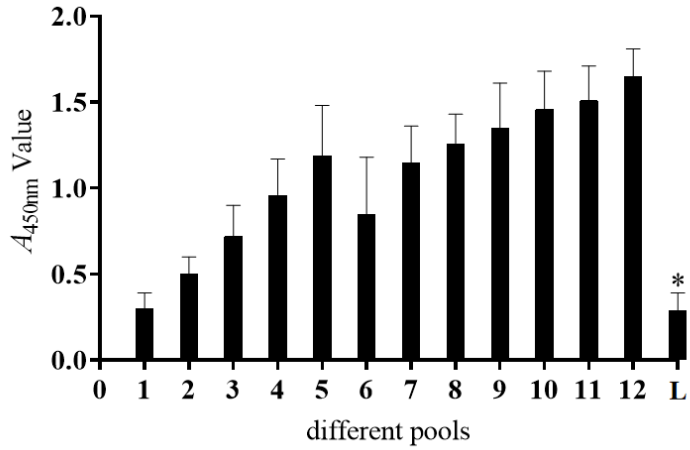

Figure 2. Comparison of relative binding force among different pools: Horizontal coordinates 1 to 12 represent Round 1 to Round 12 libraries, and L represents the initial library. *P <0.05, represents the comparison of the relative binding force between the initial library and the 2nd to 12th library.

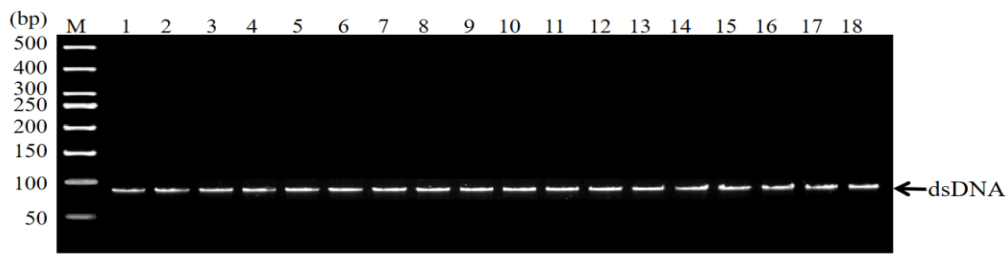

Figure 3. Identification of positive clones by PCR
Arrow: Aptamer double-stranded DNA amplified by PCR from 18 randomly selected monoclonal colonies (88nt)

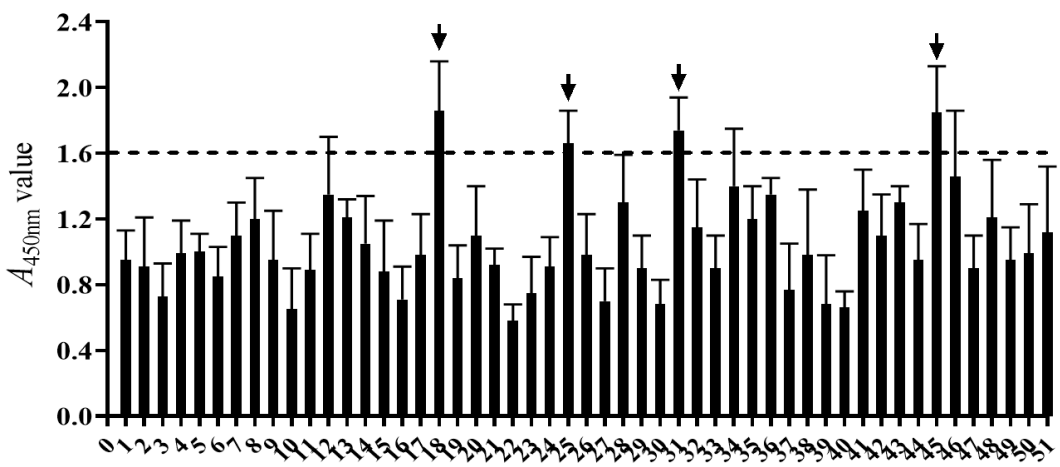

Figure 4. Comparison of relative binding force among different monoclonal aptamers
Arrow: Aptamer double-stranded DNA amplified by PCR from 18 randomly selected monoclonal colonies (88nt).

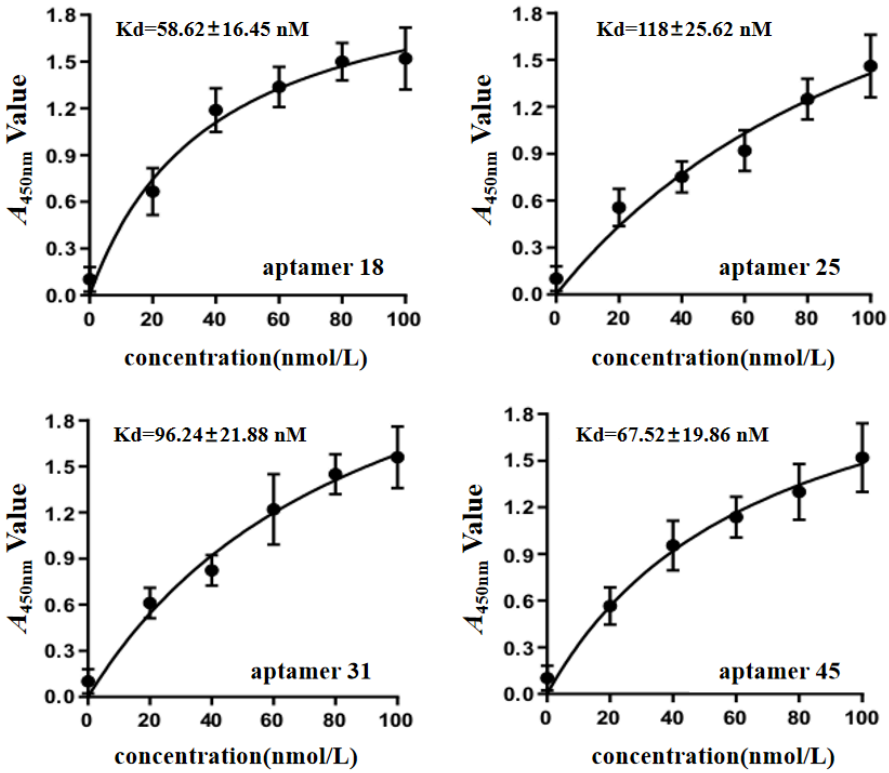

Figure 5. Detection of binding force from four kinds of monoclonal aptamers

sequences, and the results showed that they had high homology (Table 2). After using Mfold software and ELONA to analyse, we found the four aptamers may recognize the same epitope (Figure 6, Figure 7). Flow cytometry results showed that aptamer 18 demonstrated high binding force with mouse skeletal muscle myoblastic cell line C3H in a dose-dependent manner (Figure 8). By HPLC, the retention time of 5-HT standard was 4.830min. We compared the concentration of 5-HT in the culture system initially added with 200ng/ml 5-HT after 12h incubation with and without aptamer. According to the calculation of peak area, the addition of aptamer can effectively affect the reduction of 5-HT concentration in the system (Figure 9).

The mouse tail suspension model verified its antidepressant effect

The tail suspension swing time of the aptamer 18 injection group with a concentration of 80 nmol/L was much higher than that of the untreated model group and the normal saline control model group ($P < 0.05$) (Figure 10).

DISCUSSION

Early tricyclic drugs and current SSRIs antidepressants play an important role in the treatment of depression, but their side effects on cardiovascular can't be ignored.[21] Based on the above shortcomings of depression treatment drugs, this study designed a ligand that could

Table 2: Homology analysis of aptamer sequences of four relatively high binding forces

Aptamer NO.	Homologous sequence from N30		
18	…GCAA…	……GGTAGCGTTAGT…	…TTCAA……
25	…CAAG…	……GGTAGCGTTAGT…	……TCAAG……
31	…GCAAG…	……GGTAGCGTTA……	……CAAG……
45	…CAAG…	……GGTAGCGTTAGT…	……AA……

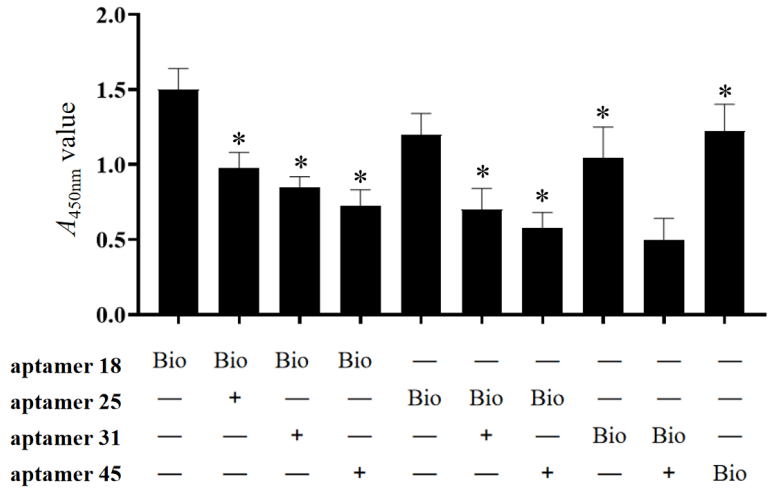

Figure 6. Verification of binding site consistency among four kinds of aptamers Bio: Adding biotin-labeled aptamers; *P<0.05:compare with different groups.

specifically bind to 5-HT_{1A}R, which blocked the uptake of HT1 through the combination of specific spatial conformation with 5-HT_{1A}R, to maintain the concentration of HT1 and achieve the effect of anti-depression. There are many kinds of HT related receptors, among which there are many studies on the correlation between 5-HT_{1A}R and depression, and the mechanism is clear.[22,23] Shioda *et al*. showed that dopamine D2L receptor deficiency leads to stress susceptibility of serotonergic neurons through 5-HT1A receptor dysfunction.[24] Sun *et al*. found the asymmetric total synthesis and identification of tetrahydroprotoberberine derivatives as novel antipsychotics with multiple effects of dopamine D(1), D(2), and 5-hydroxytryptamine 5-HT(1A).[25] Schreiber *et al*. found that improving cognition in schizophrenia with antipsychotics that elicit neurogenesis through 5-HT(1A) receptor activation.[26] Therefore, 5-HT(1A) receptor may be a potential target for antidepressant treatment. In this study, 5-HT_{1A}R was cloned and expressed as a target for the first time, and the nucleic acid aptamers with high affinity were screened by

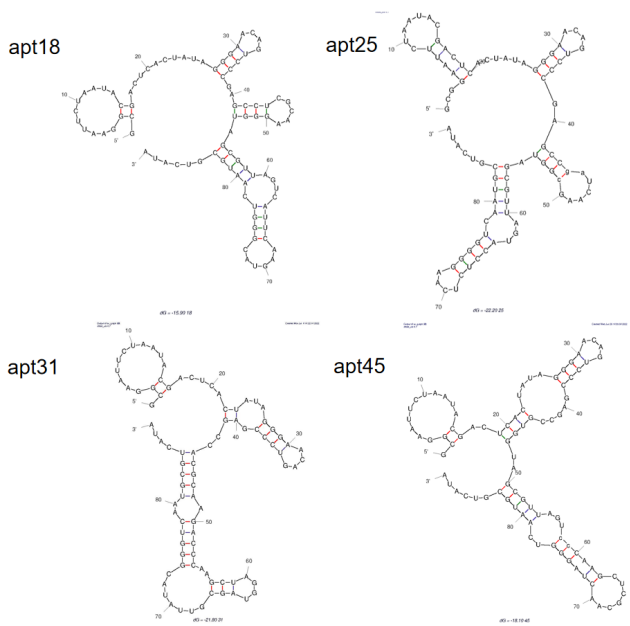

Figure 7. Secondary structure simulation diagram of aptamers

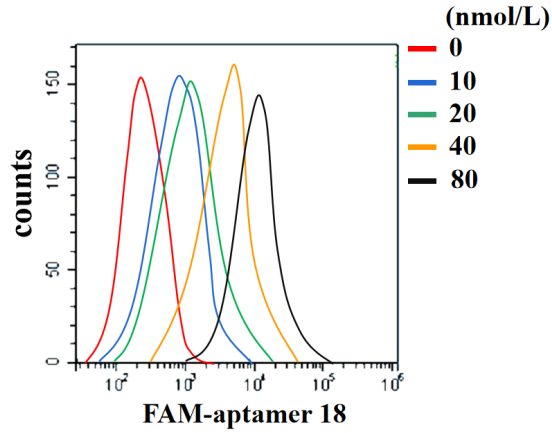

Figure 8. Analysis of binding force between aptamer 18 and C3H cell line by FMC

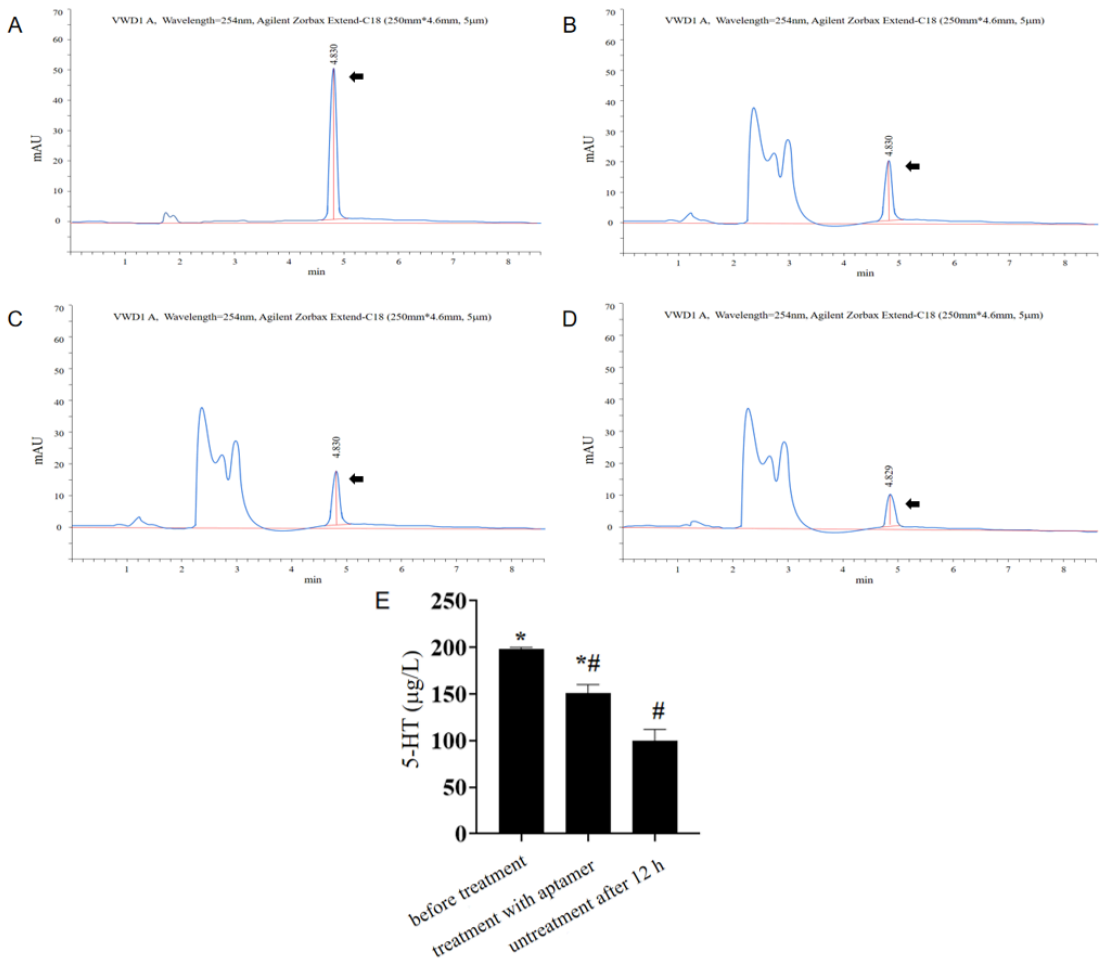

Figure 9. Detection of the 5-HT was blocked into cells by aptamer using HPLC
(A): 5-HTstandard substance; (B):Concentration of 5-HT add into culture system; (C) :Concentration of 5-HT add into culture system with Aptamer 18 blocking after 12h; (D): Concentration of 5-HT add into culture system without treatment after 12h; (E): Comparison concentration of 5-HT add into culture system with Aptamer 18 treatment before and after 12h; **P*<0.05: compare with group without treatment; #*p*<0.05: compare with group before treatment .

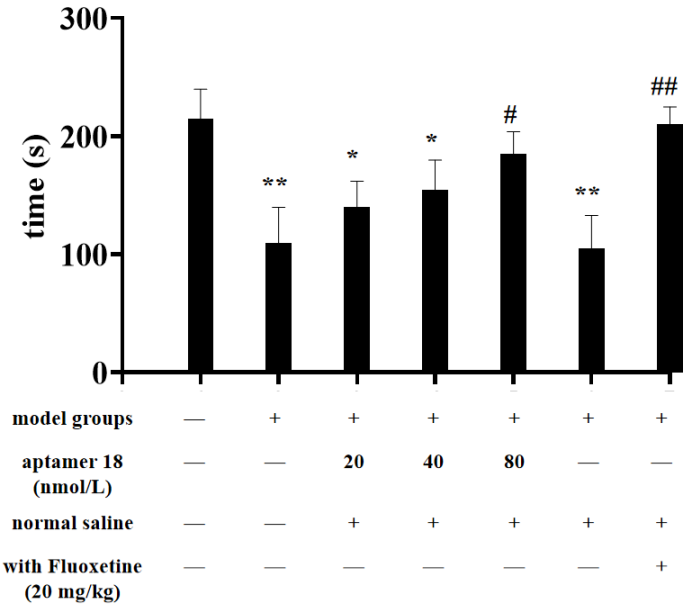

Figure 10. Comparison of exercise time from different treatment in tail suspension test
*P<0.05, **P<0.01: compare with blank control group without treatment;
# P<0.05, ##P<0.01: compare with model group without treatment.

SELEX technology, and the aptamers were used to intervene the reuptake of 5-HT. At present, nucleic acid aptamer screening has been widely used in the fields of pharmacy, detection, anti-infection and so on. It has attracted much attention because of its small molecular weight, wide target range, low immunogenicity, stable structure and easy modification.[27] Through this method, we successfully constructed and expressed 5-HT_{1A}R protein, and screened two aptamers with high specificity and high binding ability. The results showed that their binding ability was nM level. After cell reverse screening, it has almost no significant binding to other components on the cell surface. Therefore, the aptamer obtained by this screening has the potential to block 5-HT_{1A} receptor to play an anti-depressant role. In vitro experiments, it showed that aptamer could affect the concentration of 5-HT in the system, but its concentration still decreased significantly after a period of culture. It may be due to the uptake of 5-HT by other receptors.[28,29] This study only screens and intervenes for 5-HT_{1A}R, which is not enough to completely affect the concentration of 5-HT. Therefore, this study will explore the combination of other receptors in the later stage.

In subsequent animal tail suspension experiments, the activity time of aptamer injection group was much longer than that of control group (model group and normal saline group), which is equivalent to the data of drug treatment group. This result indicated that the aptamer obtained from this screening had some antidepressant effect. However, it was found that the high-dose injection of aptamer caused continuous muscle tension in mice. It is speculated that it may be because the aptamer blocked the uptake of 5-HT, resulting in frequent excitement caused by the accumulation of a large amount of 5-HT.[30] The aptamer binding 5-HT_{1A}R not only acts on the central nervous system but also has high binding ability to 5-HT_{1A}R in other parts with the same structure. Therefore, how the aptamer can only block 5-HT_{1A}R in the central nervous system without binding to 5-HT_{1A}R in the peripheral nervous system is an urgent problem to be solved. According to some specific self-assembly properties of aptamers.[31,32] 5-HT_{1A}R nucleic acid aptamers that can more accurately identify the human central nervous system is designed to obtain accurate aptamer positioning, so as to improve the application of aptamers in the treatment of depression.

In conclusion, the aptamers of 5-HT_{1A}R were obtained by SELEX technology, and the feasibility and therapeutic effect of this experiment were verified by in vitro and animal experiments. At the same time, it also provided a new idea for the research of small molecule blocking biological agents. The application of this aptamer in the

treatment of depression through 5-HT1AR May provide new ideas for drug development. However, the application of aptamers in antidepressant treatment is only basic research, and more time and funds are needed for clinical application.

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DISCLOSURE

Ethics: All animals were kept in a pathogen-free environment. The procedures for care and use of animals were approved by the Ethics Committee of the Wannan medical college, and all applicable institutional and governmental regulations concerning the ethical use of animals were followed.

Date availability: The data and material presented in this manuscript is available from the corresponding author on reasonable request.

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Conflict of interests: None

REFERENCES

1. Singkhorn O, Apidechkul T, Pitchalard K, *et al*. Prevalence of and factors associated with depression in the hill tribe population aged 40 years and older in northern Thailand. *Int J Ment Health Syst* 2021; 15(1):62. DOI: 10.1186/s13033-021-00487-7.
2. Buckman JEJ, Underwood A, Clarke K, *et al*. Risk factors for relapse and recurrence of depression in adults and how they operate: A four-phase systematic review and meta-synthesis. *Clin Psychol Rev* 2018; 64:13-38. DOI: 10.1016/j.cpr.2018.07.005
3. Greene RD, Cook A, Nowaskie D, Wang S. Neurological changes and depression: 2020 update. *Clin Geriatr Med* 2020; 36(2):297-313. DOI: 10.1016/j.cger.2019.11.009
4. Kaviani M, Nikooyeh B, Zand H, Yaghmaei P, Neyestani TR. Effects of vitamin D supplementation on depression and some involved neurotransmitters. *J Affect Disord* 2020; 269:28-35. DOI: 10.1016/j.jad.2020.03.029
5. Wang HQ, Wang ZZ, Chen NH. The receptor hypothesis and the pathogenesis of depression: Genetic bases and biological correlates. *Pharmacol Res* 2021; 167:105542. DOI: 10.1016/j.phrs.2021.105542
6. Liu X, Hao J, Yao E, *et al*. Polyunsaturated fatty acid supplement alleviates depression-incident cognitive dysfunction by protecting the cerebrovascular and glymphatic systems. *Brain Behav Immun* 2020; 89:357-70. DOI: 10.1016/j.bbi.2020.07.022
7. Suchting R, Tirumalajaru V, Gareeb R, *et al*. Revisiting monoamine oxidase inhibitors for the treatment of depressive disorders: A systematic review and network meta-analysis. *J Affect Disord* 2021; 282:1153-60. DOI: 10.1016/j.jad.2021.01.021
8. Metts AV, Rubin-Falcone H, Ogden RT, *et al*. Antidepressant medication exposure and 5-HT(1A) autoreceptor binding in major depressive disorder. *Synapse* (New York, NY) 2019;73(6):e22089. DOI: 10.1002/syn.22089
9. Micheli L, Ceccarelli M, D'Andrea G, Tirone F. Depression and adult neurogenesis: Positive effects of the antidepressant fluoxetine and of physical exercise. *Brain Res Bull* 2018;143:181-93. DOI: 10.1016/j.brainresbull.2018.09.002
10. Nanclares C, Gameiro-Ros I, Méndez-López I, *et al*. Dual antidepressant duloxetine blocks nicotinic receptor currents, calcium signals and exocytosis in chromaffin cells stimulated with acetylcholine. *J Pharmacol Exp Ther* 2018; 367(1):28-39. DOI: 10.1124/jpet.118.250969
11. Moncrieff J. Persistent adverse effects of antidepressants. *Epidemiol Psychiatr Sci* 2019;29:e56. DOI: 10.1017/S2045796019000520
12. Wang SM, Han C, Bahk WM, et al. Addressing the side effects of contemporary antidepressant drugs: A comprehensive review. *Chonnam Med J* 2018;54(2):101-12. DOI: 10.4068/cmj.2018.54.2.101
13. Kohlberger M, Gadermaier G. SELEX: Critical factors and optimization strategies for successful aptamer selection. *Biotechnol Appl Biochem* 2022;69(5):1771-92. DOI: 10.1002/bab.2244
14. Ma W, Zhan Y, Zhang Y, Mao C, Xie X, Lin Y. The biological applications of DNA nanomaterials: current challenges and future directions. *Signal Transduct Target Ther* 2021;6(1):351. DOI: 10.1038/s41392-021-00727-9
15. Song Z, Mao J, Barrero RA, Wang P, Zhang F, Wang T. Development of a CD63 aptamer for efficient cancer immunochemistry and immunoaffinity-based exosome isolation. *Molecules* 2020;25(23):5585. DOI: 10.3390/molecules25235585.
16. Zhu L, Zhao J, Guo Z, *et al*. Applications of aptamer-bound nanomaterials in cancer therapy. *Biosensors* 2021;11(9):344. DOI: 10.3390/bios11090344
17. Sinha K, Mukhopadhyay CD. Quantitative detection of neurotransmitter using aptamer: From diagnosis to therapeutics. *J Biosci* 2020;45:44. DOI: 10.1007/s12038-020-0017-x
18. Chakravarthy M, AlShamaileh H, Huang H, *et al*. Development of DNA aptamers targeting low-molecular-weight amyloid-β peptide aggregates in vitro. *Chem Commun* (Camb) 54(36):4593-96. DOI: 10.1039/c8cc02256a
19. Tang XL, Wu SM, Xie Y, *et al*. Generation and application of ssDNA aptamers against glycolipid antigen ManLAM of Mycobacterium tuberculosis for TB diagnosis. *J Infect* 2016;72(5):573-86. DOI: 10.1016/j.jinf.2016.01.014

20. Tang XL, Zhou YX, Wu SM, Pan Q, Xia B, Zhang XL CFP10 and ESAT6 aptamers as effective mycobacterial antigen diagnostic reagents. *J Infect* 2014;69(6):569-80. DOI: 10.1016/j.jinf.2014.05.015
21. Calvi A, Fischetti I, Verzicco I, *et al*. Antidepressant drugs effects on blood pressure. *Front Cardiovasc Med* 2021;8:704281. DOI: 10.3389/fcvm.2021.704281
22. Narváez M, Andrade-Talavera Y, Valladolid-Acebes I, *et al*. Existence of FGFR1-5-HT1AR heteroreceptor complexes in hippocampal astrocytes. Putative link to 5-HT and FGF2 modulation of hippocampal gamma oscillations. *Neuropharmacology* 2020;170:108070. DOI: 10.1016/j.neuropharm.2020.108070
23. Gorinski N, Bijata M, Prasad S, *et al*. Attenuated palmitoylation of serotonin receptor 5-HT1A affects receptor function and contributes to depression-like behaviors. *Nat Commun* 2019;10(1):3924. DOI: 10.1038/s41467-019-11876-5
24. Shioda N, Imai Y, Yabuki Y, *et al*. Dopamine D2L receptor deficiency causes stress vulnerability through 5-HT1A receptor dysfunction in serotonergic neurons. *J Neurosci* 2019;39(38):7551-63. DOI: 10.1523/JNEUROSCI.0079-19.2019
25. Sun H, Zhu L, Yang H, *et al*. Asymmetric total synthesis and identification of tetrahydroprotoberberine derivatives as new antipsychotic agents possessing a dopamine D(1), D(2) and serotonin 5-HT(1A) multi-action profile. *Bioorg Med Chem* 2013;21(4):856-68. DOI: 10.1016/j.bmc.2012.12.016
26. Schreiber R, Newman-Tancredi A. Improving cognition in schizophrenia with antipsychotics that elicit neurogenesis through 5-HT(1A) receptor activation. *Neurobiol Learn Mem* 2014;110:72-80. DOI: 10.1016/j.nlm.2013.12.015
27. He F, Wen N, Xiao D, *et al*. Aptamer-based targeted drug delivery systems: Current potential and challenges. *Curr Med Chem* 2020;27(13):2189-219. DOI: 10.2174/0929867325666181008142831
28. Maxwell J, Gleason SD, Falcone J, *et al*. Effects of 5-HT(7) receptor antagonists on behaviors of mice that detect drugs used in the treatment of anxiety, depression, or schizophrenia. *Behav Brain Res* 2019;359:467-73. DOI: 10.1016/j.bbr.2018.11.019
29. Polovinkin L, Hassaine G, Perot J, *et al*. Conformational transitions of the serotonin 5-HT(3) receptor. *Nature* 2018;563(7730):275-9. DOI: 10.1038/s41586-018-0672-3
30. Zaitsu K, Noda S, Iguchi A, *et al*. Metabolome analysis of the serotonin syndrome rat model: Abnormal muscular contraction is related to metabolic alterations and hyper-thermogenesis. *Life Sci* 2018;207:550-61. DOI: 10.1016/j.lfs.2018.06.031
31. Ran XQ, Qian HL, Yan XP. Aptamer self-assembly-functionalized nanochannels for sensitive and precise detection of chloramphenicol. *Anal Chem* 2021;93(42):14287-92. DOI: 10.1021/acs.analchem.1c03396
32. Zhang L, Wang M, Zhu Z, *et al*. A GD2-aptamer-mediated, self-assembling nanomedicine for targeted multiple treatments in neuroblastoma theranostics. *Mol Ther Nucleic Acids* 2021;26:732-48. DOI: 10.1016/j.omtn.2021.08.021