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Research Article

Domesticated HERV-W env contributes to the activation of the small conductance Ca^{2+}-activated K^+ type 2 channels via decreased 5-HT4 receptor in recent-onset schizophrenia

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ABSTRACT

The human endogenous retroviruses type W family envelope (HERV-W *env*) gene is located on chromosome 7q21-22. Our previous studies show that HERV-W env is elevated in schizophrenia and HERV-W env can increase calcium influx. Additionally, the 5-HTergic system and particularly 5-hydroxytryptamine (5-HT) receptors play a prominent role in the pathogenesis and treatment of schizophrenia. 5-hydroxytryptamine receptor 4 (5-HT4R) agonist can block calcium channels. However, the underlying relationship between HERV-W env and 5-HT4R in the etiology of schizophrenia has not been revealed. Here, we used enzyme-linked immunosorbent assay to detect the concentration of HERV-W env and 5-HT4R in the plasma of patients with schizophrenia and we found that there were decreased levels of 5-HT4R and a negative correlation between 5-HT4R and HERV-W env in schizophrenia. Overexpression of HERV-W env decreased the transcription and protein levels of 5-HT4R but increased small conductance Ca^{2+}-activated K^+ type 2 channels (SK2) expression levels. Further studies revealed that HERV-W env could interact with 5-HT4R. Additionally, luciferase assay showed that an essential region (−364 to −176 from the transcription start site) in the SK2 promoter was required for HERV-W env-induced SK2 expression. Importantly, 5-HT4R participated in the regulation of SK2 expression and promoter activity. Electrophysiological recordings suggested that HERV-W env could increase SK2 channel currents and the increase of SK2 currents was inhibited by 5-HT4R. In conclusion, HERV-W env could activate SK2 channels via decreased 5-HT4R, which might exhibit a novel mechanism for HERV-W env to influence neuronal activity in schizophrenia.

1. Introduction

As remnants of ancient retroviral infections, human endogenous retroviruses (HERVs) become part of the host genome and comprise up to 8% of the human genome (Kury et al., 2018). The general structure of a full-length HERV proviral sequence includes gag, pol, and env, flanked by the 5′ and 3′ long-terminal repeats (LTRs), as same as exogenous retroviruses (Grandi and Tramontano, 2018). Most HERVs have accumulated mutations and deletions, rendering them inactive and preventing them as infectious viruses (Hughes and Coffin, 2002). However, a growing number of HERVs have full-length open reading frames (ORF) and encode functional retroviral proteins, serving several vital functions that include placental syncytiotrophoblasts formation, antiviral immune defense, and gene transcription (Mao et al., 2021; Grandi and Tramontano, 2018; Li and Karlsson, 2016). In addition, environmental factors and infectious agents, may potently re-activate HERVs transcription, including caffeine and aspirin (Liu et al., 2013), human cytomegalovirus (Assinger et al., 2013), hepatitis B virus (Liu et al., 2017), human immunodeficiency virus 1 (Srinivasachar et al., 2020), or influenza A viruses (Nellaker et al., 2006). Several lines of evidence show that HERVs have been implicated in the pathology of certain cancers (Yu et al., 2014; Zhou et al., 2021), autoimmune diseases (Kremer et al., 2019), and neuropsychiatric disorders (Perron et al., 2012; Huang et al., 2006; Suntsova et al., 2015). HERVs are divided into families based on sequence similarities (Jern et al., 2005), of which HERV-W has been extensively studied and researched. Recent studies report that there is an

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elevated level of the HERV-W envelope (HERV-W env) in schizophrenia, and HERV-W env seems to be a factor that contributes to the development of schizophrenia (Huang et al., 2006; Karlsson et al., 2004; Yao et al., 2008). Nevertheless, the pathogenic mechanisms of HERV-W env in the context of schizophrenia remain still unclear.

Schizophrenia is a severe mental illness that affects about 1% of the population worldwide (Marder and Cannon, 2019). Biochemical theories suggest that schizophrenia occurs mainly due to unbalanced neurotransmitters in the brain, including dopamine, glutamate, serotonin, and acetylcholine (Yan et al., 2022; Cooper et al., 1992; McCutcheon et al., 2020a; Scarr et al., 2009). Although the dopamine hypothesis has become the most influential hypothesis of schizophrenia for a long time, attention has been refocused on the 5-hydroxytryptamine (5-HT) hypothesis of schizophrenia due to the minimal effect of antipsychotic drugs targeted by DRD2 on negative symptoms of schizophrenia (Mishara and Goldberg, 2004). Currently, available therapies for schizophrenia are mostly based on dopaminergic and serotonergic (5-HT) theories (Wang et al., 2018a; Koblan et al., 2020; Devroye et al., 2018). The 5-HT hypothesis of schizophrenia postulates that abnormal 5-HT metabolism, involving 5-HT receptors and transporters, might contribute to schizophrenia (Juza et al., 2020; WOOLLEY and CAMPBELL, 1962; Kulikova and Kulikov, 2019). There is increasing evidence showing that 5-HT receptors (5-HTRs) have been implicated in the pathophysiology of schizophrenia. Among 5-HTRs, the 5-HT4 receptor (5-HT4R) is of considerable interest because it modulates the release of several neurotransmitters and bidirectionally impacts memory formation. Additionally, several lines of evidence reveal that the haplotype in 5-HT4R is significantly associated with schizophrenia (Suzuki et al., 2003; Ohtsuki et al., 2002), and 5-HT4R agonists can improve cognitive function (Teixeira et al., 2018; Lamirault and Simon, 2001; Kumar et al., 2017). Intriguing, accumulating pieces of evidence reveal that the 5-HT4R inhibits K^+ current in neurons via activation of a cyclic AMP-dependent protein kinase (Mlinar et al., 2006; Ansanay et al., 1995). Small conductance Ca^{2+}-activated K^+ (SK) channels, including the SK2 channel, are modulated by 5-HT receptors (Guo et al., 2021; Grunnet et al., 2004). SK channels (SK1–SK3) are activated and increase intracellular Ca^{2+} concentration in response to Ca^{2+} influx or Ca^{2+} release (Ngo-Anh et al., 2005). It should be noted that HERV-W env evokes Ca^{2+} (Chen et al., 2019) and activates the SK3 channel (Li et al., 2013), which contributes to cognitive symptoms of people with schizophrenia due to the CAG repeat polymorphism (Grube et al., 2011). However, there is no report on the relationship among HERV-W env, SK2 channels, and 5-HT4R.

In this study, we discovered that 5-HT4R was lower in plasma from individuals with recent-onset schizophrenia than in healthy people. Further analysis identified a negative correlation between the HERV-W env and 5-HT4R in schizophrenia patients. Cytological experiments indicated HERV-W env augmented SK2 expression associated with depressed expression of 5-HT4R. Furthermore, a promoter region (from −364 to −176) was required for HERV-W env-induced SK2 expression. We also gave proof that HERV-W env could activate the SK2 channel and the activator of SK2 channels was regulated by 5-HT4R. SK2 channels might dynamically regulate neuronal excitability and synaptic transmission. It would provide a new mechanism whereby HERV-W env contributed to the etiology of schizophrenia.

2. Materials and methods

2.1. Patients and healthy controls

We collected blood samples (plasma samples) from 64 individuals, which composed of 33 schizophrenia patients (median age of 41, ranging from 20 to 66 years) and 31 health controls (median age of 44, ranging from 23 to 65 years), from Wuhan Mental Health Center (see details in Supplementary Tables S1 and S2). All patients conformed to the symptoms with recent-onset schizophrenia-related psychoses as defined by the Diagnostic and Statistical Manual of Mental Disorders, 5th edition. All patients were admitted to the hospital for the first time, and none had medical treatment before admission. We excluded patients who had acute infectious, inflammatory, or neurological diseases. By screening examination, none of them had neurological or psychiatric disorders. All plasma samples were mixed with ethylenediaminetetraacetic acid and stored at −80 °C immediately until experiments.

2.2. ELISA

We obtained the plasma samples from each subject. We diluted plasma samples 2-fold, to a final volume of 50 μL per sample before experiment. Plasma levels of 5-HT4R and HERV-W env were analyzed by commercial enzyme-linked immunosorbent assay (ELISA) kits [human 5-hydroxytryptamine receptor 4 ELISA Kit (Warner Bio, Wuhan, China) and human syncytin-1 (HERV-W env) ELISA Kit (Warner Bio, Wuhan, China)], according to the manufacturer's protocols. After the reaction, the absorbance was detected at 450 nm with an enzyme-labelled instrument (Thermo Multiskan™ FC, USA). Finally, standard curves were plotted, and the concentrations of 5-HT4R and HERV-W env were calculated according to the manufacturer's instructions.

2.3. Phylogenetic analysis

To study the role of HERV-W env in the evolution, we collected 42 retrovirus amino acid sequences, including exogenous and endogenous retroviruses (See in Supplementary Table S3). The MEGA software was used for sequence alignment. Multiple alignments were performed by ClustalW with default settings. We used IQ-Tree (Chen et al., 2022) to construct the HERV-W env phylogeny tree by using the maximum likelihood (ML) and bootstrap analysis was carried out with ultrafast bootstrap (1000 replicates). The best-fit model of the phylogeny tree was WAG + F + G4.

2.4. Plasmids and plasmids constructs

The plasmids pCMV- HERV-W env (Huang et al., 2011), pxj40-HA-HERV-W env (Yan et al., 2022), pEGFP-HERV-W env-TM (Wang et al., 2018b), and pIRES2-EGFP- HERV-W env (Li et al., 2013) were obtained as previously described. For the luciferase assay, the full-length SK2 promoter (−572 to +90) and various 5′- truncated SK2 promoter fragments (truncation 1, -364 to +90; truncation 2, -175 to +90) were cloned into the pGL3-basic vector. Besides, two special promoter fragments truncation 3 (−572 to −365) and truncation 4 (−364 to −176) were constructed to study the promoter regions regulated by HERV-W env. All the primers were described in Supplementary Table S4. Overexpression plasmid of human 5-HT4R was purchased from Vigene Biosciences (termed named pENTER-5-HT4R/pENTER-Flag-5-HT4R).

2.5. Cell culture and transfection

We purchased the human neuroblastoma cell line SH-SY5Y and human embryonic kidney cell line HEK293T from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cell line SHSY5Y was maintained in a 1:1 mixture of Minimum Essential Media (Gibco, USA) and F12 Medium (Gibco, USA) containing 100 mmol/L sodium pyruvate (Gibco, USA). The HEK293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM). All cell culture media were supplemented with 10% fetal bovine serum (Gibco, USA), and 100 U/mL penicillin-streptomycin liquid (Gibco, USA) at 37 °C with 5% CO_2. Plasmids were transfected into cells using Turbofect transfection reagent (Thermo, USA) according to the manufacturer's instructions. After transfection for 48 h, we collected the cells for further study.

2.6. Real-time quantitative PCR

Total RNA was extracted from 1 × 10^6 cells using TRIzol reagent (Invitrogen, USA). The cDNA was subsequently synthesized using ReverTra Ace qPCR RT Master Mix with gDNA Remover kit according to the manufacturer's instructions (Toyobo, Japan). Real-time qPCR was performed on the Mini Opticon Detect (Bio-Rad, USA) using Thermo Scientific Strips of 8 Tubes and SYBR Green qPCR Master Mix (Invitrogen). GAPDH was used as a housekeeping gene to normalize gene expression by the $2^{-\Delta\Delta Ct}$ method (ΔΔCt = ΔCt (a target sample) −ΔCt (a reference sample), ΔCt = Ct (Target)−Ct (GAPDH)). The primers of SK2, 5-HT4R, and GAPDH had been designed by primer 3 plus online software and NCBI Primer-BLAST. All primers were listed in Supplementary Table S5. The primer concentrations used for RT-qPCR was 3.3 μmol/L and the PCR program was described as follows: 95 °C for 3 min, followed by 45 cycles at 95 °C for 45 s, 56 °C for 45 s, and 72 °C for 45 s.

2.7. Western blotting

The proteins were isolated from SH-SY5Y cells (1 × 10^6) using RIPA reagents (Pierce, USA) supplemented with protease and phosphatase inhibitors (Roche Applied Science, Indianapolis, USA). Proteins were separated by 12% SDS-PAGE and electroblotted onto PVDF membranes (Millipore, USA). The membranes were subsequently incubated with primary antibodies: anti-SK2 (1:1000, ab99457, Abcam, USA), anti-5HT4 Receptor (1:1000, ab60359, Abcam, USA), anti-HERV-W env (1:1000, ab179693, Abcam, USA), rabbit anti DDDDK-Tag pAb (1: 2500, AE004, Abclonal, China), mouse anti DDDDK-Tag mAb (1: 2500, AE005, Abclonal, China), anti HA-Tag mAb (1: 2500, AE008, Abclonal, China), Rabbit anti HA-Tag pAb (1: 2500, AE036, Abclonal, China) and anti-GAPDH (1:10,000, ab8245, Abcam, USA) with 5% nonfat dry milk in TBST. After incubating overnight, the secondary antibodies (HRP-conjugated anti-rabbit/mouse) were added to the membrane and then visualized with an ECL kit (Millipore, USA) and analyzed with a chemiluminescence gel imaging system (Amersham, Sweden).

2.8. Co-immunoprecipitation assay

For the co-immunoprecipitation assay, the cells were plated in a 10 cm dish at 1 × 10^7 cells, and cultured until 80%–90% confluent. SH-SY5Y and HEK293T cells co-transfected with the plasmid pxj40-HA-HERV-W env and pENTER-Flag-5-HT4R. After 48 h transfection, cells were collected and lysed with IP lysis buffer. The HA, Flag, and IgG antibodies were used to pull down the protein complex. Pierce Protein A/G Plus Agarose (Thermo Scientific, United States) was added to the IP complexes solutions and incubated overnight at 4 °C. The HERV-W env/5-HT4R complex levels were analyzed by Western blotting. The following antibodies were used in this study: HA (Abcam, ab236632, dilution 1:100), Flag (Abcam, ab205606, dilution 1:100), and IgG antibodies (Abcam, ab172730, dilution 1:100). IgG was used as a negative control. The experiment was performed independently three times.

2.9. Confocal microscopy

Cells (5 × 10^3 cells) were seeded in each confocal dish. Triple-immunofluorescence signals were examined using a confocal laser scanning microscope (TCS SP8, Leica Microsystems) using the HCX PL APO 63*/1.40 oil objective lens. The plasmid pEGFP- HERV-W env-TM and pENTER-5-HT4R were co-transfected into SH-SY5Y and HEK293T cells. The collected images were projected and analyzed onto the two-dimensional planes using a display of green (GFP, excitation spectrum: 488 nm), red (Cy3, excitation spectrum: 552 nm), and blue (DAPI, spectrum excitation: 405 nm). The experiment was repeated three times.

2.10. Luciferase activity assay

The cells were incubated in 24-well plates (2.5 × 10^5 cells/well). After transfecting for 24 h, cells were lysed, and luciferase activity was performed using the Promega Dual-Luciferase Reporter Assay (Promega, USA) according to the manufacturer's instructions. All experiments were repeated three times.

2.11. Electrophysiology

We used the cells SH-SY5Y to study the SK2 channel currents by whole-cell patch-clamp recordings. Transfected cells were seeded at low density onto glass and incubated for 2 h at 37 °C, 5% CO_2 in air, and 80% relative humidity. The internal pipette and external solutions were prepared according to the procedures reported previously (Mizukami et al., 2015). Briefly, the patch pipette (borosilicate glass) was filled with a solution containing: 144 mmol/L potassium gluconate, 1.15 mmol/L $MgCl_2$, 5 mmol/L EGTA, 10 mmol/L HEPES, and 500 mmol/L Ca^{2+} free (pH 7.25 with KOH). The external solution consisted of: 140 mmol/L NMG, 4 mmol/L KCl, 1 mmol/L $MgCl_2$, 5 mmol/L glucose, and 10 mmol/L HEPES (pH 7.4 with HCl). We performed the experiments at room temperature (24 or 25 °C) and clamped the membrane potential at +60 mV for 50 ms followed by ramps from −120 mV to +70 mV for 500 ms. Currents were recorded by using an EPC-9 amplifier (HEKA; Lambrecht, Germany). Data were analyzed using Fitmaster software (HEKA Electronics), Igor Pro software (WaveMetrics), and origin8.

In whole-cell experiments, the following drugs were supplied into external solutions: Apamin (SK2 channel inhibitor, 100 nmol/L), Lei-Dab7 (SK2 channel selected inhibitor, 50 μmol/L), and RS67333 (5-HT4R agonist, 50 μmol/L).

2.12. Statistical analysis

For the clinical results, we used median and nonparametric analysis. The relationship between HERV-W env and 5-HT4R was performed by correlation analysis.

The data were shown as mean ± SD (standard deviation). All experiments were repeated three times. GraphPad Prism 5, Origins 8, and SPSS software were used for statistical analyses. The $P < 0.05$ was considered statistically significant.

3. Results

3.1. Decreased 5-HT4R plasma levels and a negative correlation between 5-HT4R and HERV-W env in recent-onset schizophrenia

5-HT4R is one of the 14 subtypes that constitute the serotonin receptor family. The haplotype of 5-HT4R is associated with schizophrenia (Ohtsuki et al., 2002). Here, we measured the expression of 5-HT4R in the plasma samples (consisting of 33 patients and 31 healthy controls). The demographic and clinical characteristics of recent-onset schizophrenia and healthy controls were shown in Supplementary Table S2. There was no significant difference in age, gender distribution, level of education, smoking status, and body mass index (BMI) between the schizophrenia and controls. Our results implied that the plasma 5-HT4R concentration was significantly lower in recent-onset schizophrenia than in healthy subjects by ELISA assay (Fig. 1A and B, Table 1). Consistent with our previous studies, plasma HERV-W env level was significantly higher in schizophrenia than in healthy controls (Fig. 1C and D, Table 2) (Wang et al., 2018b; Huang et al., 2011). Furthermore, the correlation analysis through linear regression revealed a negative correlation between 5-HT4R and HERV-W env protein in recent-onset schizophrenia (Fig. 1E; $R^2 = 0.7635$). Interestingly, we found the levels of 5-HT4R below the normal range (5-HT4R < 9744.654 ng/L), termed as 5-HT4R (−), were detected in 18/20 (90%) HERV-W env (+) (HERV-W env >1900.802 ng/L) schizophrenia patients, while 5-HT4R (+) (5-HT4R ≥

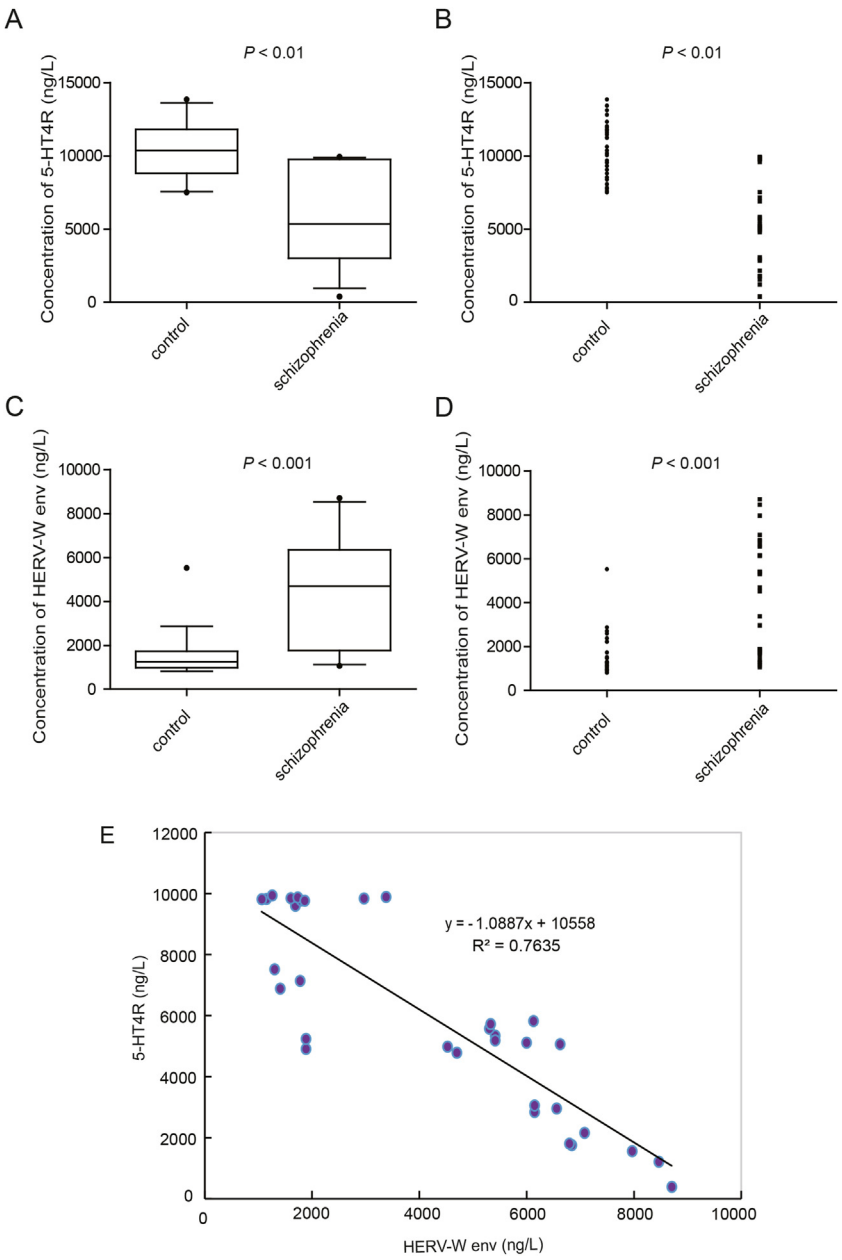

Fig. 1. The expression levels of 5-HT4R and the correlation between 5-HT4R and HERV-W env in recent-onset schizophrenia. (**A**) and (**B**) The concentration of 5-HT4R in healthy controls (N = 31) and schizophrenia (N = 33) by ELISA. (**C**) and (**D**) The concentration of HERV-W env in healthy controls (N = 31) and schizophrenia (N = 33) by ELISA. **E** Linear regression correlation between HERV-W env and 5-HT4R expression levels in schizophrenia. X-axis: the concentration of HERV-W env; Y-axis: the concentration of 5-HT4R. The line represents the calculated "best-fit" equation of values within the boxed area, with correlation value indicated on the top ($R^2 = 0.76$). $P < 0.01$, $P < 0.001$ by median and nonparametric analysis. Note: each point generally represents a single patient, but a few are overlapping and cannot be separated on the graph.

Table 1
The concentration of 5-HT4R in the blood of controls and schizophrenia patients.

Control (N = 31, ng/L)		Schizophrenia (N = 33, ng/L)	
Mean	10425.247	Mean	5911.396
Median	10370.948	Median	5345.387
Std.Deviation	1855.475	Std.Deviation	3110.668
Skewness	0.018	Skewness	−0.035
Sta. Error of Skewness	0.421	Sta. Error of Skewness	0.409
Range	6359.1	Range	9551.12
Minimum	7503.12	Minimum	382.79
Maximum	13862.22	Maximum	9933.92

Table 2
The concentration of HERV-W env in the blood of controls and schizophrenia patients.

Control (N = 31, ng/L)		Schizophrenia (N = 33, ng/L)	
Mean	1555.3	Mean	4268.005
Median	1244.589	Median	4696.970
Std.Deviation	941.928	Std.Deviation	2496.592
Skewness	2.787	Skewness	0.117
Sta. Error of Skewness	0.421	Sta. Error of Skewness	0.409
Range	4707.79	Range	7640.69
Minimum	811.69	Minimum	1060.61
Maximum	5519.48	Maximum	8701.3

Table 3
The consistency of HERV-W env and 5-HT4R concentration in schizophrenia patients.

Schizophrenia patients	HERV-W env (+)	HERV-W env (−)	Consistency ratio
5-HT4R (+)	2	8	79%
5-HT4R (−)	18	5	

HERV-W env (+): the expression of HERV-W env above 1900.802 ng/L; HERV-W env (−): the expression of HERV-W env below 1900.802 ng/L; 5-HT4R (+): the expression of 5-HT4R above 9744.654 ng/L; 5-HT4R (−): the expression of 5-HT4R below 9744.654 ng/L.

9744.654 ng/L) were detected in 8/13 (62%) HERV-W env (−) (HERV-W env ≤1900.802 ng/L) schizophrenia patients (Table 3), suggesting that the expression of 5-HT4R and HERV-W env was consistency. All evidence indicated that plasma 5-HT4R concentration was notably decreased and showed a negative correlation between 5-HT4R and HERV-W env in schizophrenia.

3.2. HERV-W env depressed the expression of 5-HT4R in SH-SY5Y cells

HERV-W belongs to class I HERVs, which is among the oldest group of HERVs and has been extensively researched (Grandi and Tramontano, 2017; Griffiths, 2001). To investigate the evolutionary relationship of HERV-W and other retroviruses, a phylogenetic tree was constructed based on the envelope protein, including 42 protein sequences (class I, class II, class III HERVs, gammaretroviruses, deltaretroviruses, betaretroviruses, alpharetroviruses, and lentiviruses) by using Maximum Likelihood (ML) analysis. Phylogenetic tree analysis revealed that HERV-W env was closely related to HERV9 and HERVH families, and displayed similar branch lengths, suggesting a similar age of evolution (Fig. 2A). Previous studies reported HERV-H emerged in the genome over 30 million years ago (Sverdlov, 2000), indicating a long evolutionary history of HERV-W env. Our results revealed a negative correlation between HERV-W env and 5-HT4R in recent-onset schizophrenia. Therefore, human neuroblastoma cell line SH-SY5Y was used to further explore the causal relationship between HERV-W env and 5-HT4R in schizophrenia. Successful transfection was verified by a blunt increase of HERV-W env mRNA and protein levels in the transfected cells (Supplementary Fig. S1). Results suggested HERV-W env dramatically decreased the mRNA expression level of 5-HT4R (Fig. 2B). The result of subsequent Western blot experiments was consistent with the mRNA level change of 5-HT4R (Fig. 2C). Those results demonstrated that HERV-W env could down-regulated the expression of 5-HT4R in SH-SY5Y.

3.3. 5-HT4R and HERV-W env physically interacted at the plasma membrane of cells

To investigate whether 5-HT4R could interact with HERV-W env, we performed co-immunoprecipitation assays (COIP) in SH-SY5Y cells and HEK293T cells. HA-HERV-W env and Flag-5-HT4R were co-immunoprecipitated and analyzed by western blotting. Results suggested that 5-HT4R protein (or HERV-W env protein) was detected after the anti-HA antibody (anti-Flag antibody) was used to immunoprecipitate HERV-W env-5-HT4R complex in SH-SY5Y cells, and no band was detected when IgG was used to immunoprecipitate (Fig. 2D, lane 3, lane 4, and lane 2). Similar results had been observed in HEK293T cells (Supplementary Fig. S2A, lane 3, lane 4, and lane 2). These data indicated that HERV-W env could directly interact with 5-HT4R.

Furthermore, the laser confocal scanning microscope was used to study the cellular localization of HERV-W env and 5-HT4R by the co-transfection of pEGFP-HERV-W env-TM plasmid containing the transmembrane domain of HERV-W env and pENTER-5-HT4R plasmid in SH-SY5Y and HEK293T separately (Fig. 2E and Supplementary Fig. S2B). Results revealed that both HERV-W env-TM and 5-HT4R were located in the membrane of SH-SY5Y (see Fig. 2E) and HEK293T (Supplementary Fig. S2B) cells. We found a high rate of colocalization of HERV-W env with 5-HT4R in SH-SY5Y (54.16%, Fig. 2E) and HEK293T cells (66.57%, Supplementary Fig. S2B). Taking together, HERV-W env and 5-HT4R constitutively interacted at the plasma membrane of cells.

3.4. HERV-W env upregulated the expression of SK2 via increasing its promoter activity

The alteration of function and expression of the small conductance calcium-activated potassium channel (SK channel) is involved in the activity of 5-HT neurons. Electrophysiological recordings reveal that 5-HT increases excitability and burst firing by reducing SK currents (Deemyad et al., 2011). SK2 can regulate neuronal excitability and modulate hippocampal learning, memory, and synaptic plasticity (Hammond et al., 2006). Moreover, SK2 is associated with cognitive impairments, a core symptom of schizophrenia. Therefore, we tried to discover the effect of HERV-W env on the expression of SK2 in SH-SY5Y. The transfection efficiency of HERV-W env had been verified by real-time qPCR and Western blotting (Supplementary Fig. S1). There was a significantly higher expression of SK2 mRNA in the pCMV-HERV-W env-transfected cells than in the control groups (Fig. 3A). Similarly, the protein levels were increased in the pCMV-HERV-W env-transfected cells compared to control groups (Fig. 3B). It suggested HERV-W env could upregulate SK2 expression.

Typically, promoter plays essential roles in regulating gene expression. To determine whether HERV-W env has distinct implications for SK2 promoter activity, a genomic DNA fragment of the SK2 promoter region (range from −572 to +90) was isolated and ligated to the luciferase reporter vector (see diagram in Fig. 3C). As shown in Fig. 3D, HERV-W env could increase the activity of SK2 promoter by about 1.5 folds (pCMV as a control group, $P < 0.001$), demonstrating HERV-W env could activate SK2 promoter.

Next, a series of truncations were generated to identify the location of the binding site (see diagram in Fig. 3C). T1, T2, T3, and T4 indicated truncated promoters −364 to +90, −175 to +90, −572 to −365, and −364 to −176, respectively. T1 promoter activity was significantly higher than the control (Fig. 3E), indicating that the regions from −572 to −365 lacked the promoter activity. Subsequent studies confirmed the inference by showing that T3 promoter activity had no significant difference compared with controls (Fig. 3F). Furthermore, the luciferase activity assay showed that T2 was not a functional region in SK2 promoter (Fig. 3E). Therefore, we hypothesized the functional regions might be located from −364 to −176. Further studies revealed that T4 (from −364 to −176) was the minimum sequence required in the HERV-W env-enhanced SK2 promoter activity (Fig. 3F). These results suggested that HERV-W env regulated SK2 transcription by modulating its promoter activity at the location from −364 to −176.

3.5. 5-HT4R was involved in the regulation of SK2 expression

The previous study suggests that 5-HT4R can influence the synaptic membrane potential via G-protein signaling cascades to regulate the open state of downstream SK channels (Eglen et al., 1995). Plasmid pENTER-5-HT4R or 5-HT4R agonist (RS67333) was used to unveil the effect of 5-HT4R on the expression of SK2. Successful transfection was verified by real-time qPCR and Western blotting (Supplementary Fig. S3 and Supplementary Fig. S4). We found that 5-HT4R could reverse the increase of SK2 levels induced by HERV-W env in SH-SY5Y cells (Fig. 4). The results from real-time qPCR (Fig. 4A) and Western blotting (Fig. 4B) showed that the mRNA and protein levels of SK2 in the HERV-W env transfection cells were increased by 1.4 times ($P < 0.01$) and 0.7 times ($P < 0.05$), compared with the control groups. The increased levels of SK2 were dramatically decreased when HERV-W env was co-transfected with 5-HT4R. Similarly, we got the same results when using the 5-HT4R agonist RS67333 (Fig. 4C and D). The expression of SK2 was increased both in transcription (Fig. 4C) and protein levels (Fig. 4D), while it was

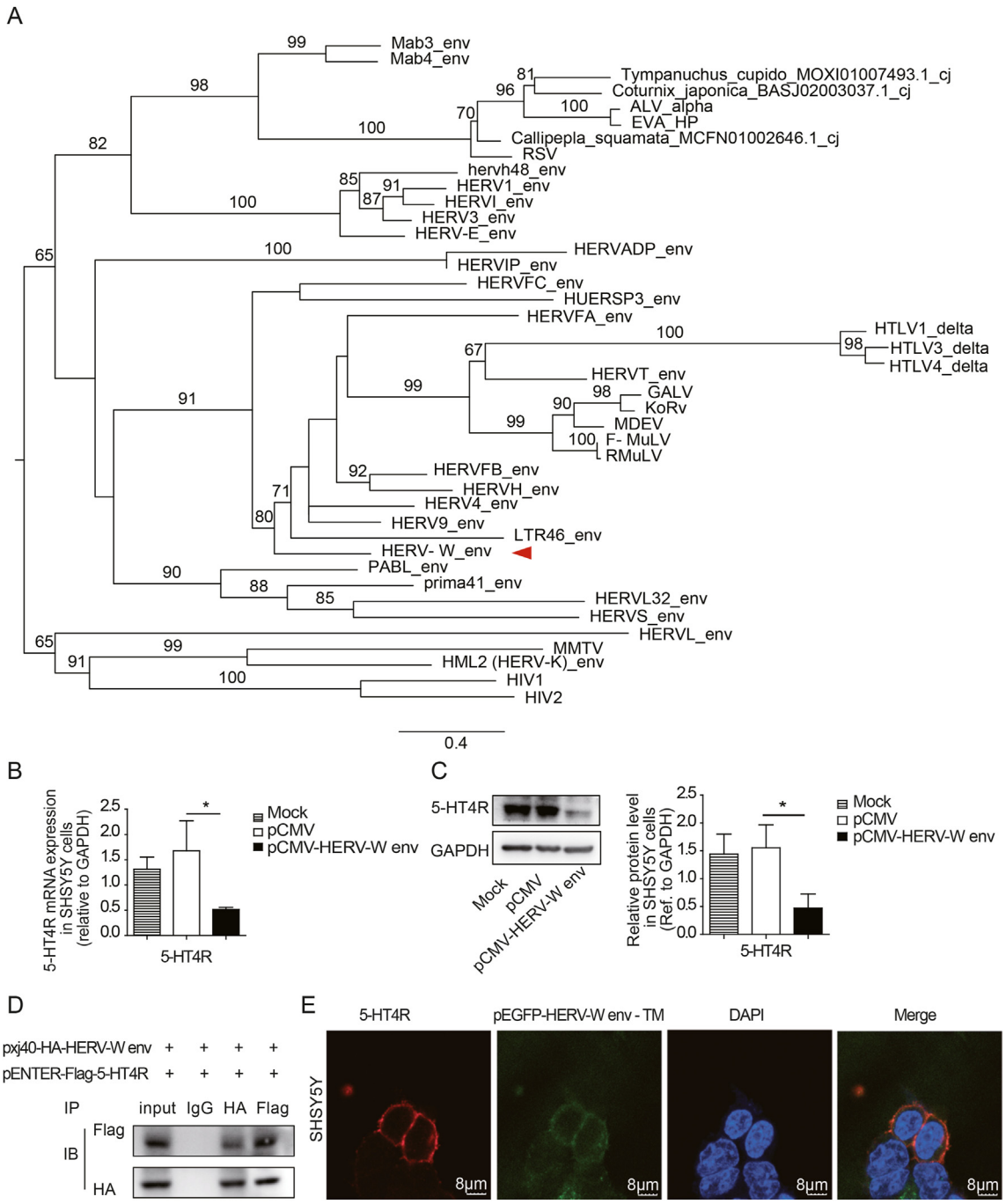

Fig. 2. HERV-W env could down-regulate the expression of 5-HT4R and directly interact with 5-HT4R in human neuroblastoma SH-SY5Y cells. **A** HERV-W env phylogenetic tree. Phylogenetic tree constructed using amino acid sequences of the envelope proteins consensuses region of HERVs and other representative retroviruses (alpha-, beta-, gamma-, deltaretroviruses, and lentiviruses). HERVs family contained class I (HERV-H env, HERV9 env, HERV-W env, HERVADP env, HERV-T env, HERVIP env, HERVFA_env, HERVFB_env, HERV-FC_env, hervh48_env, prima41 env, HERV1 env, HERV3 env, HERV4_env, HERVI env, HERV-E env, PABL env, and HUERSP3_env), class II (HERVL_env and HML2_env), and class III (HERVL32_env and HERVS_env). Alpharetrovinus: ALV (Chicken), EVA_HP (Chicken), Cja_-BASJ02003037.1 (*Coturnix japonica*), RSV (Chicken), Tcu_MOXI01007493.1 (Tympanuchus cupido), and Csq_MCFN01002646.1 (*Callipepla squamata*). Betaretrovirus: MMTV (mouse). Gammaretrovirus: F MuLV (mouse), R-MuLV (mouse), MDEV (mouse), KoRV (*Phascolarctos cinereus*), Mab3_env (Mabuya), Mab4_env (Mabuya), and GALV (Gibbon). Deltaretrovirus: HTLV1 (human), HTLV3 (human), and HTLV4 (human). Lentivirus: HIV1 (human) and HIV2 (human). Unclassifiable: LTR46_env (human). The red triangle is the HERV-W env. The env tree is rooted in betaretrovirus and lentiretrovirus. **B–C** SH-SY5Y cells were transfected with pCMV-HERV-W env or empty vector pCMV. The mRNA and protein levels of 5-HT4R were determined by real-time quantitative PCR (**B**) and Western blotting (**C**). **D** Coimmunoprecipitation assays (COIP) were performed with anti-HA-HERV-W env or anti-Flag-5-HT4R antibodies by Western blotting in SH-SY5Y cells. **E** STED images of HERV-W env-TM (green) and 5-HT4R (red). All experiments were repeated three times. Data are presented as the mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA). $^*P < 0.05$.

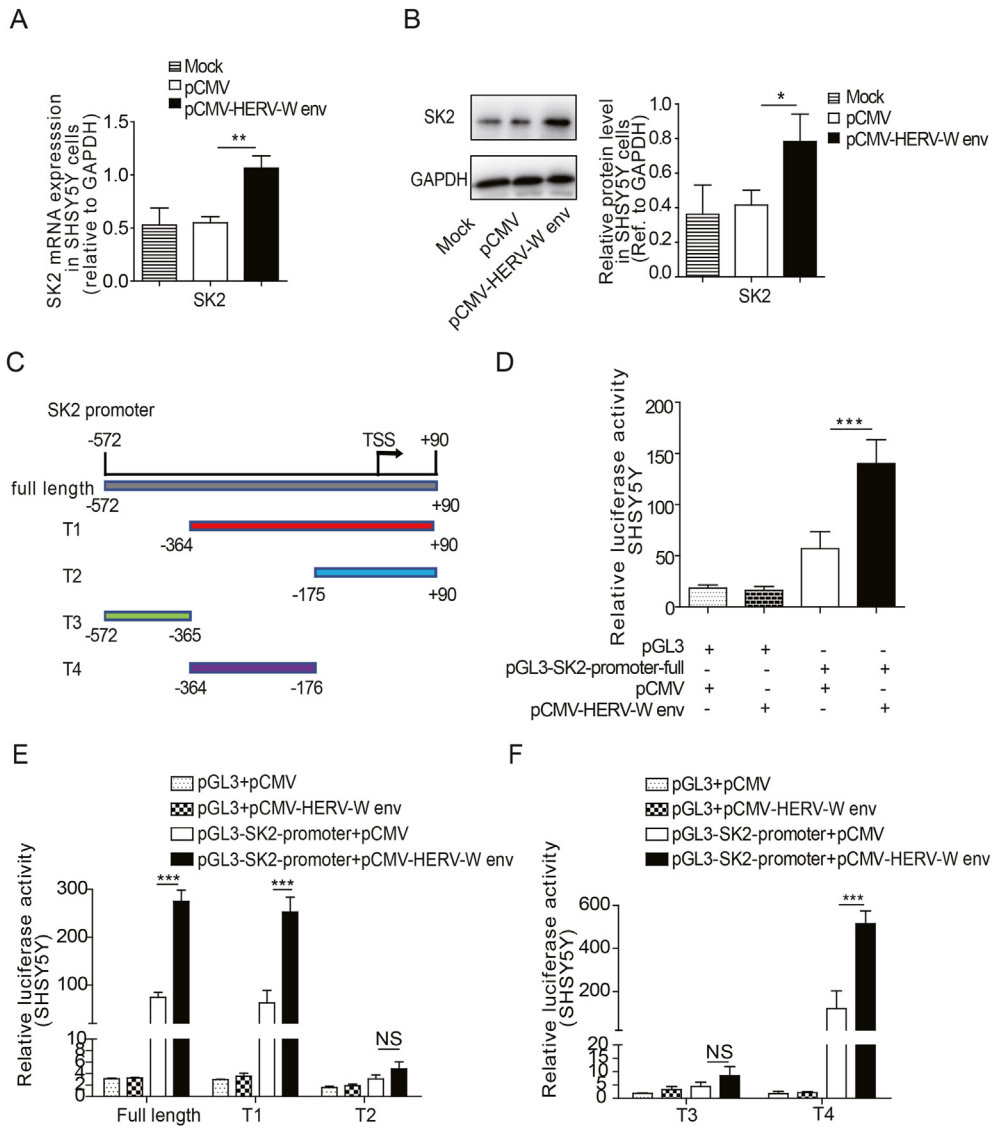

Fig. 3. HERV-W env positively regulated the SK2 expression by enhancing its promoter activity. (**A, B**) SH-SY5Y cells were transfected with pCMV-HERV-W env or empty vector pCMV. The mRNA and protein levels of SK2 were determined by real-time quantitative PCR (**A**) and Western blotting (**B**). **C** Schematic illustration of SK2 promoter constructs. SK2 full-length promoter region from −572 to +90. T1-T2, serial 5′ truncated SK2 promoter constructs. T3-T4, two special promoter fragments. (**D–F**) Luciferase activity for SK2 promoter. **D** SH-SY5Y cells were cotransfected with full-length SK2 promoter-luciferase reporter construct and HERV-W env expression vector or empty vector pCMV. (**E–F**) Reporter plasmids containing different lengths of putative SK2 promoters were cotransfected with pCMV-HERV-W env or empty vector pCMV in SHSY5Y cells. Each experiment was repeated three times. Data shown are mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{NS}P > 0.05$.

inhibited in the presence of 5-HT4R agonist RS67333. These results above all suggested that HERV-W env increased the expression of SK2 via deregulating of the 5-HT4R.

In addition, a dual-luciferase reporter assay had been used to study the role of 5-HT4R in the regulation of SK2 promoter activity. Briefly, SH-SY5Y cells were tri-transfected with SK2 promoter-luciferase reporter, HERV-W env, and 5-HT4R expression vector (Fig. 4E). Interestingly, the increased promoter activity of SK2 by HERV-W env was substantially attenuated in the presence of the 5-HT4R. Taken together, 5-HT4R participated in HERV-W env-regulated SK2 expression.

3.6. HERV-W env activated SK2 channels in SH-SY5Y

SK2 channels have an important role in the regulation of membrane excitability. Overexpression of SK channels in animals has an impact on learning and memory (Hammond et al., 2006). To determine the effect of HERV-W env on SK2 channel function, here we performed whole-cell patch-clamp experiments in SH-SY5Y cells. Fig. 5A showed the representative current traces recording at 500 nmol/L free Ca^{2+} in the internal pipette solution. The currents were obtained at a holding potential of −60 mV, voltage steps ranging from −120 mV to +70 mV with 500 ms duration. Apamin was a remarkably selective SK channel blocker (Sailer et al., 2002; Adelman et al., 2012). Therefore, 100 nmol/L Apamin was added to the chamber solution. As shown in Fig. 5, SK2 channel currents were higher in the HERV-W env overexpression cell than in the pIRES2-EGFP-control cell (Fig. 5A and B). The currents were dramatically inhibited when adding Apamin to the chamber solution. To further confirm our results, Lei-dab7, another selective SK2 channel blocker was also added to measure the current. Consistently, the currents were decreased after treating with 50 μmol/L Lei-dab7 (Fig. 5A and B). Additionally, currents were normalized by cell membrane capacitance to correct the cell size difference. As we can see in the

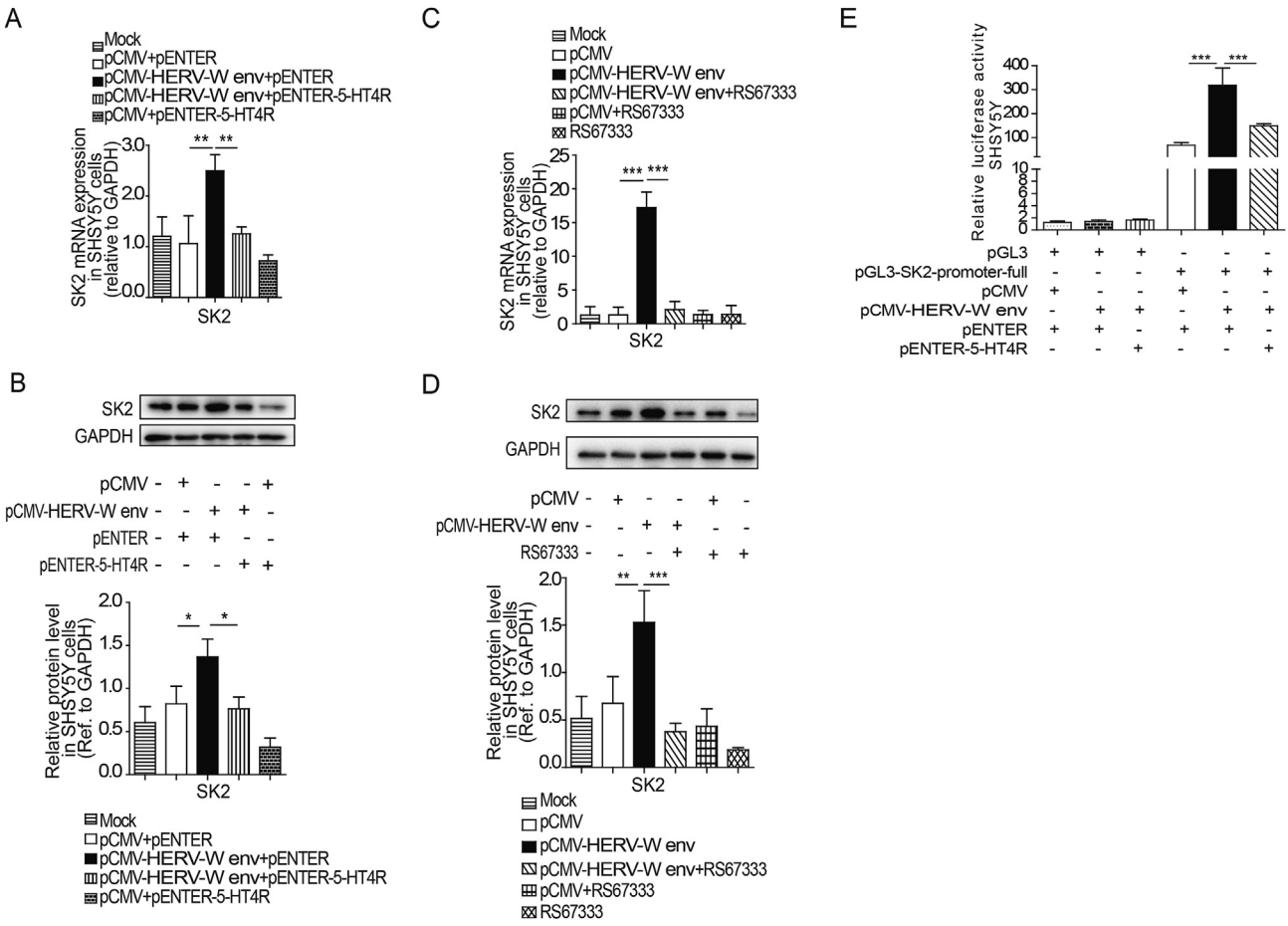

Fig. 4. 5-HT4R mediated the up-regulation of SK2 induced by HERV-W env. SH-SY5Y cells were transfected with HERV-W env and pENTER-5-HT4R. The expression of the SK2 mRNA was examined by RT-qPCR (**A**). The expression of SK2 protein was detected by Western blotting (**B**). SH-SY5Y cells were transfected with HERV-W env and then treated by 5-HT4R agonist RS67333. The expression level of the SK2 mRNA and protein was determined by RT-qPCR (**C**) and Western blotting (**D**). **E** Assessment of 5-HT4 receptor function in regulating the increased SK2 promoter activity via tri-transfection of pGL3/pGL3-SK2-promoter-full, pCMV/pCMV-HERV-W env plasmid, and pENTER/pENTER-5-HT4R. Data shown are mean ± SD from three independent experiments. Statistical analysis: one-way ANOVA (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Fig. 5C, the average current density (pA/pF) was separately increased by approximately 1.2, 1.1 and 1.1 folds at 50 mV, 60 mV and 70 mV in HERV-W env overexpression cells (102.0 ± 4.2 pA/Pf, 115.3 ± 6.3 pA/pF, 133.0 ± 10.6 pA/pF) compared to pIRES2-EGFP transfected control groups (47.2 ± 1.4 pA/pF, 54.4 ± 3.9 pA/pF, 62.5 ± 5.3 pA/pF) ($P < 0.001$, $P < 0.01$, $P < 0.01$) (Fig. 5C). After treatment with the SK2 channel blockers Apamin or Lei-dab7, the currents were significantly decreased (37.2 ± 10.2 pA/pF, 42.0 ± 14.4 pA/pF, 47.4 ± 13.8 pA/pF, Apamin; 52.1 ± 10.7 pA/pF, 57.3 ± 11.7 pA/pF, 63.4 ± 16.1 pA/pF, Lei-dab7) ($P < 0.001$, $P < 0.001$, $P < 0.001$, Apamin; $P < 0.001$, $P < 0.01$, $P < 0.01$, Lei-dab7) (Fig. 5C). These results indicated that HERV-W env could induce the activation of the SK2 channel in the SH-SY5Y cell.

3.7. HERV-W env triggered SK2 channels activation through 5-HT4R signaling

To further investigate the underlying mechanism of the opening of the SK2 channel, 5-HT4R agonist RS67333 was used in external solutions. Fig. 6A and B showed the representative current traces in SH-SY5Y. Consistently, SK2 channel currents were higher in the HERV-W env overexpression cell than in the pIRES2-EGFP-control cell (Fig. 6A and B). After adding 50 μmol/L RS67333, the currents were significantly decreased by about 50% in the HERV-W env overexpression cell (Fig. 6 A, middle panel). Sequentially, the change of currents was limited by adding 100 nmol/L Apamin into the chamber solution (Fig. 6A, right panel). To further verify the result, we also used another SK2 channel blocker Lei-dab7. Fortunately, the change curves of SK2 currents were similar to Apamin (Fig. 6B). The current-voltage (I–V) curve had shown in Fig. 6C and D. Moreover, to correct the cell size difference, the SK2 channel currents were normalized by cell membrane capacitance and expressed as current density (pA/pF). Before treatment with RS67333, the average current density (pA/pF) was increased in the HERV-W env overexpression cell compared to pIRES2-EGFP transfected control cell (Fig. 6E and F). The average current density (pA/pF) was robust decreased after adding RS67333 in HERV-W env-expressing cell by approximately 0.46, 0.48 and 0.51 folds at 50 mV, 60 mV and 70 mV (52.8 ± 18.5 pA/pF, 60.0 ± 18.0 pA/pF, 63.8 ± 22.0 pA/pF) compared to the control groups (98.7 ± 10.1 pA/pF, 115.4 ± 8.4 pA/pF, 131.0 ± 8.8 pA/pF) ($P < 0.05$, $P < 0.05$, $P < 0.05$). Sequentially, the average current density (pA/pF) had no significant differences by treating with Apamin (49.7 ± 20.0 pA/pF, 52.6 ± 23.6 pA/pF, 61.6 ± 21.8 pA/pF) (see in Fig. 6E). Similarly, there was a decrease of about 0.4, 0.39, and 0.38 folds at 50 mV, 60 mV, and 70 mV in the presence of RS67333 (Fig. 6F). And then, no charge had been found by applying Lei-dab7 (Fig. 6F). Collectively, these data indicated that HERV-W env induced the activation of the SK2 channel by inhibiting the 5-HT4R in human neuroblastoma cells.

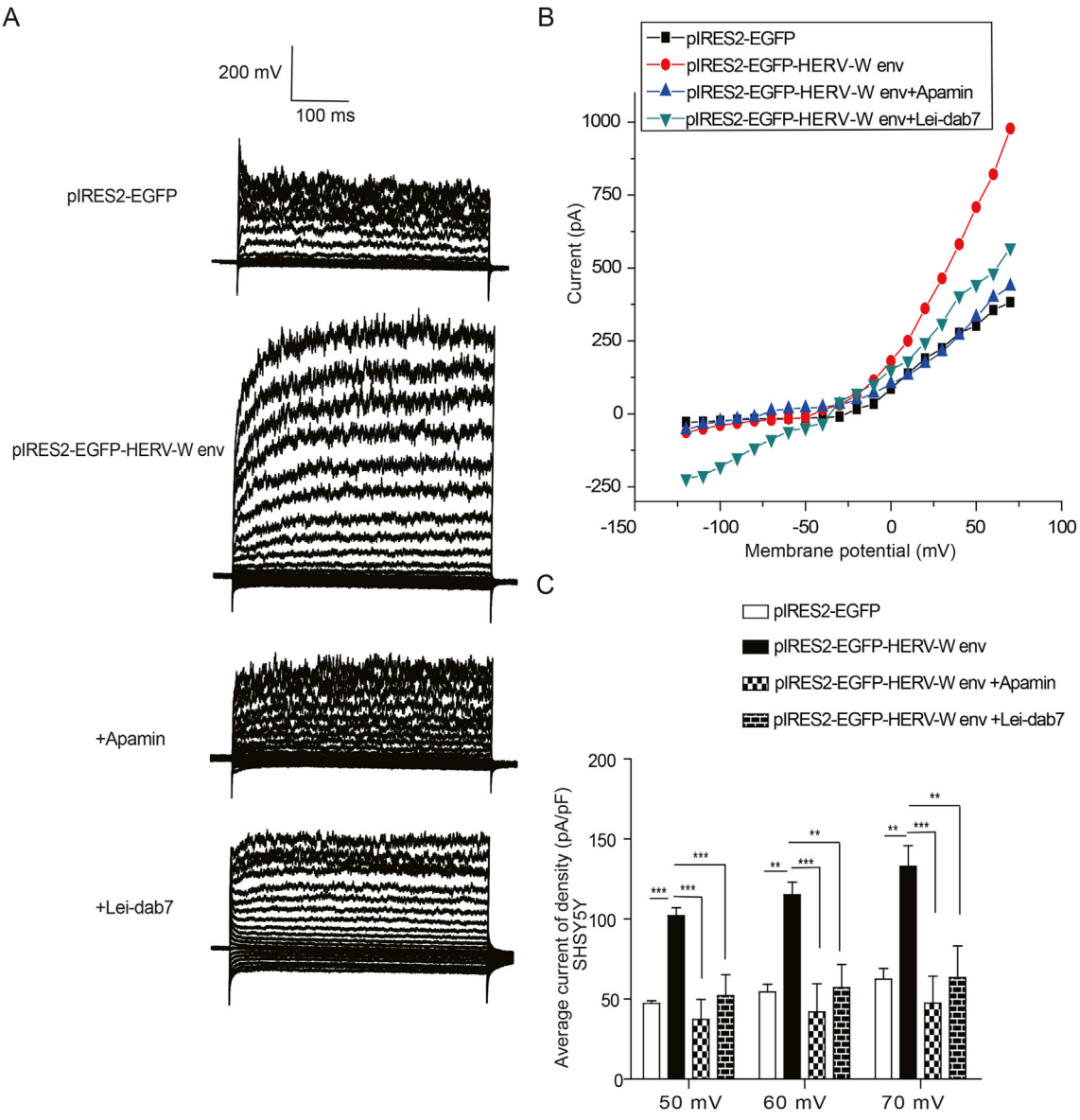

Fig. 5. HERV-W env triggered SK2 channel activation in SH-SY5Y. **A** Representative current recorded in pIRES2-EGFP (control cell) and in pIRES2-EGFP-env (HERV-W env expression cell) using whole-cell patch, voltage steps ranging from −120 mV to +70 mV in the presence of 100 nmol/L Apamin or 50 μmol/L Lei-dab7 at 500 nmol/L Ca^{2+} free. **B** The currents of SK2 in SH-SY5Y cell were measured by whole-cell patch experiments, 100 nmol/L Apamin and 50 μmol/L Lei-dab7 were separately added to the chamber solution. **C** Histograms showed the average current density (pA/pF) of SK2 channel in SH-SY5Y from pIRES2-EGFP controls, pIRES2-EGFP-HERV-W env-transfected, pIRES2-EGFP-HERV-W env-transfected + Apamin or Lei-dab7 at 50 mV, 60 mV and 70 mV. Group data are mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$; one-way ANOVA. Each experiment was repeated three times.

4. Discussion

HERV-W env, which has a critical role in receptor recognition and membrane fusion, is the first env characterized for its domestication. Aberrant expression of HERV-W env relates to several diseases, such as multiple sclerosis (Charvet et al., 2021), schizophrenia (Huang et al., 2011), and cancers (Zhou et al., 2021; Yu et al., 2014). Previous studies have demonstrated the presence of HERV-W env in the cerebrospinal fluid or the serum of schizophrenia (Perron et al., 2008; Karlsson et al., 2001). In addition, RNA sequencing analysis suggests that HERV-W env exhibits high levels of transcription in many regions of human brains with schizophrenia (Li et al., 2019). Sequentially, data from our lab have revealed an increased level of HERV-W env in recent-onset schizophrenia (Huang et al., 2011; Wang et al., 2018b). We find that HERV-W env mediates neuroinflammation, including inducing inflammatory marker C-reactive protein (CRP) through the TLR3 signal pathway (Wang et al., 2018b), increasing NO production (Xiao et al., 2017), and upregulating the expression of interleukin-10 and TNF-α by inhibiting MyD88s in glial cells (Wang et al., 2021). Further studies indicate that HERV-W env activates both the SK3 channel (Li et al., 2013) and the TRPC3 channel (Chen et al., 2019). In addition, HERV-W env induces the expression of schizophrenia risk genes, such as brain-derived neurotrophic factor (BDNF) (Huang et al., 2011) through phosphorylation of GSK3β at Ser9 (Qin et al., 2016). Recently, we also report that HERV-W env contributes to mitochondrial complex I defect (Xia et al., 2021). Many studies suggest that the overexpression of schizophrenia risk genes, the abnormality of neuroinflammation, the activator of the SK3 channel, and the impairment of mitochondria have a critical role in the pathology of schizophrenia. Thus, it's necessary to process the underlying roles of HERV-W env in the development of schizophrenia.

Schizophrenia is a complex disorder characterized by mental dysfunction, including alterations in perception, thought, and antisocial

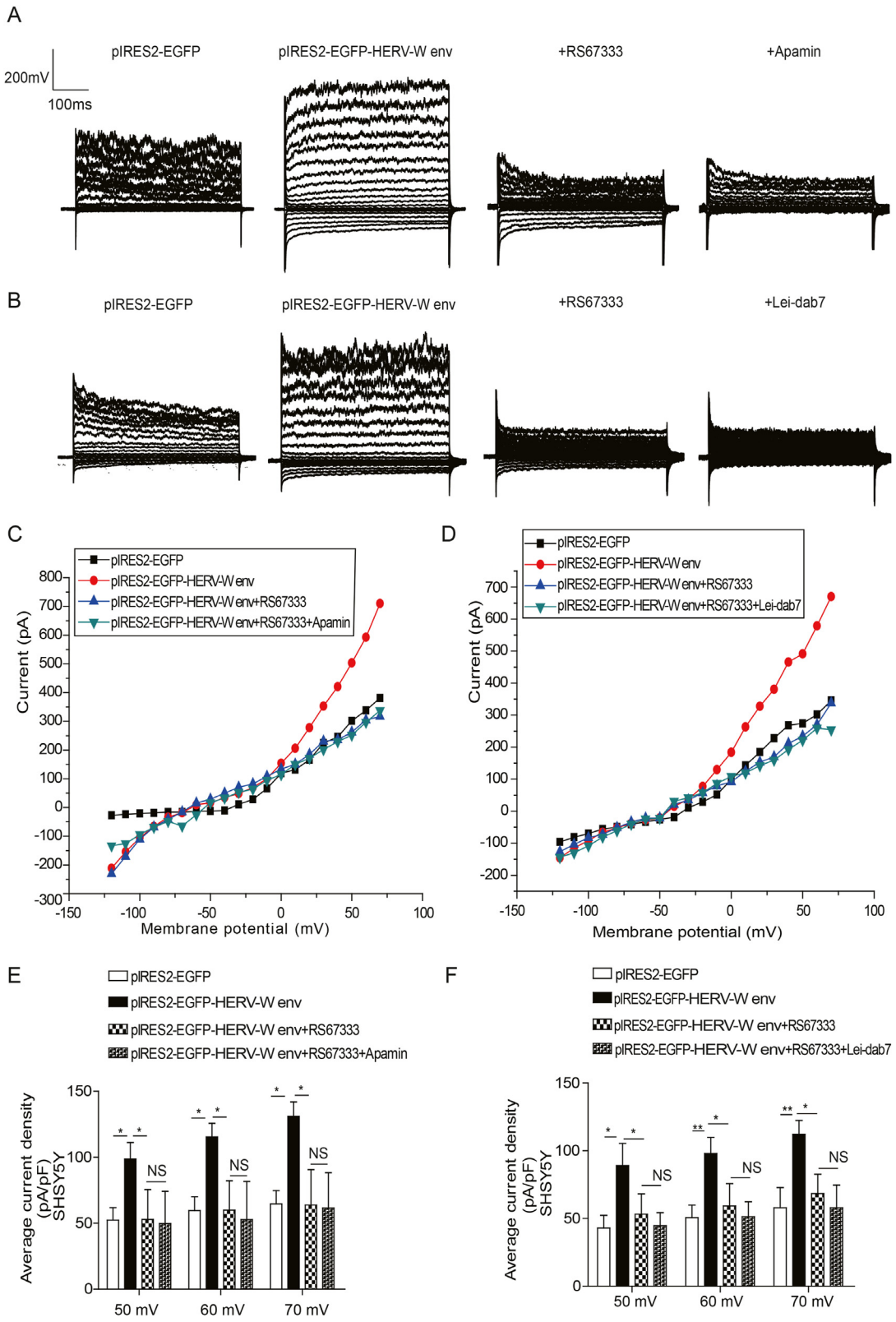

Fig. 6. HERV-W env induced SK2 channel activation by inhibiting the 5-HT4 receptor signal pathway. (**A, B**) Representative SK2 channel current traces were obtained in SH-SY5Y cells from pIRES2-EGFP (control cell), pIRES2-EGFP-HERV-W env (HERV-W env-expression cell), pIRES2-EGFP-HERV-W env in the presence of RS67333, pIRES2-EGFP-HERV-W env in the presence of RS67333 and Apamin (**A**, right), or Lei-dab7 (**B**, right). (**C, D**) The currents of SK2 in SH-SY5Y cells were measured by whole-cell patch experiments. (**C**) 100 nmol/L Apamin, (**D**) 50 μmol/L Lei-dab7. (**E, F**) Histograms showed the average current density (pA/pF) of SK2 channel in SH-SY5Y from pIRES2-EGFP control, pIRES2-EGFP-HERV-W env-transfected, pIRES2-EGFP-HERV-W env-transfected + RS67333, pIRES2-EGFP-HERV-W env-transfected + RS67333+Apamin or Lei-dab7 at 50 mV, 60 mV and 70 mV. (**E**) 100 nmol/L Apamin, (**F**) 50 μmol/L Lei-dab7. Data are presented as mean ± SD. Data represent three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{NS}P > 0.05$; one-way ANOVA.

behavior displays (McCutcheon et al., 2020b). Recently, the serotonin hypothesis has regained interest due to the benefit of the second-generation ('atypical') antipsychotic drugs (e.g., clozapine, targeting 5-HT2A receptor) in treating negative and cognitive symptoms of schizophrenia (Pere and Chaumet-Riffaud, 1990; Pallanti et al., 1999). The Hippocampus, a region of the brain, is associated with cognitive function. Hippocampal grey matter volume and functional connectivity are important for the early diagnosis of schizophrenia (Liu et al., 2020). It has been reported that reduced 5-HT4R density has a strong correlation with hippocampal volume change in first-episode psychosis (Park et al., 2021). The 5-HT4R antagonist GR113808 sufficiently blocks the potentiation of postsynaptic potential in the hippocampal CA3-CA1 synapse, suggesting 5-HT4R is related to cognitive function (Teixeira et al., 2018). The activation of 5-HT4R is necessary for maintaining the proper excitability in the hippocampus and memory formation (Teixeira et al., 2018; Karayol et al., 2021). These results indicate that the 5-HT4R may be a promising target in treating cognitive symptoms. Besides, single nucleotide polymorphism (SNP) suggests there is a significant association between 5-HT4R and schizophrenia (Suzuki et al., 2003). But to date, there is lacking evidence to elucidate the 5-HT4R concentration in the blood of first onset schizophrenia. In this paper, we reported a decreased level of 5-HT4R in the plasma of schizophrenia and found a negative correlation between 5-HT4R and HERV-W env. Deep studies suggested that HERV-W env could increase the expression of SK2 and trigger the SK2 channel via decreased 5-HT4R.

Data from our clinical research firstly showed a decreased plasma 5-HT4R level in resent-onset schizophrenia. However, the underlying relationship of HERV-W env and 5-HT4 receptors in the etiology of schizophrenia has not been revealed. Phylogenetic tree analysis showed HERV-W was related to HERV9 and HERVH families. It is consistent with the reports by Grandi and his colleague in the LTR or Pol phylogenies (Grandi et al., 2018, 2020). Accumulating evidence suggests that abnormal activation of those families may contribute to schizophrenia, indicating HERV-W has an important role in the pathology of schizophrenia (Li et al., 2019; Huang et al., 2006). Here, we found there was a negative correlation between the expression of 5-HT4R and HERV-W env in schizophrenia. HERV-W env positive patients show more manic symptoms of schizophrenia (Tamouza et al., 2021), which may accompany with cognitive impairment. Recent evidence shows that 5-HT4R agonist (RS67333) can improve cognitive symptoms in schizophrenia (Kumar et al., 2017; Abboussi et al., 2016). Interestingly, we found that HERV-W env could downregulate the level of 5-HT4R in human neuroblastoma cell lines SH-SY5Y. In addition, HERV-W env showed clear co-localization and interaction with 5-HT4R. 5-HT4R is localized in the hippocampus, which has a major role in controlling emotional and cognitive function (Tregellas et al., 2014; Delavari et al., 2021). Combined with the fact that 5-HT4R activator could improve cognitive effects, these suggested that HERV-W env might contribute to the impaired cognitive symptoms of schizophrenia by inhibiting the expression of the 5-HT4R in schizophrenia.

The 5-HT4R is a G protein-coupled receptor (GPCR). 5-HT4R activation increases neuronal excitability by GPCR signal transduction. A previous study indicates that HERV-W env could increase SK3 protein levels (Li et al., 2013). Therefore, we wanted to know whether HERV-W env has an effect on SK2 expression. We found that HERV-W env could increase SK2 transcript and protein levels. In addition, the luciferase reporter assay showed that HERV-W env could enhance SK2 promotor activity. The region (from −364 to −176) was required for HERV-W env-induced SK2 expression. And there was a CCAAT box in this region, where transcription factors can bind to control gene expression (Fritz and Kaina, 2001). A recent study suggests the transcription factor cAMP-response element binding protein (CREB) phosphorylation can enhance the SK2 promoter (Yang et al., 2021). Our previous studies show that HERV-W env can increase the phosphorylation of CREB. And p-CREB is involved in the regulation of SK3 promoter activity. Maybe, HERV-W env regulated SK2 gene expression via increasing its promoter activity at the region from −364 to −176 by regulating the binding of CREB.

Intriguing, our experiment dates suggested that 5-HT4R could convert the increase of SK2 expression induced by the HERV-W env. Moreover, 5-HT4R was involved in the regulation of SK2 promoter activity. The 5-HT4R activator could increase intracellular cAMP levels, thereby modulating the activity of protein kinase A (PKA) (Weninger et al., 2014; Park et al., 2021). Furthermore, PKA activity has previously been implicated in the reorganization of SK2 surface expression (Ren et al., 2006). Therefore, we proposed that HERV-W env upregulated SK2 expression via the 5-HT4 receptor signaling pathway.

Increased expression of SK2 might contribute to the open of SK2 channels. SK2 channels are widely expressed in the central nervous

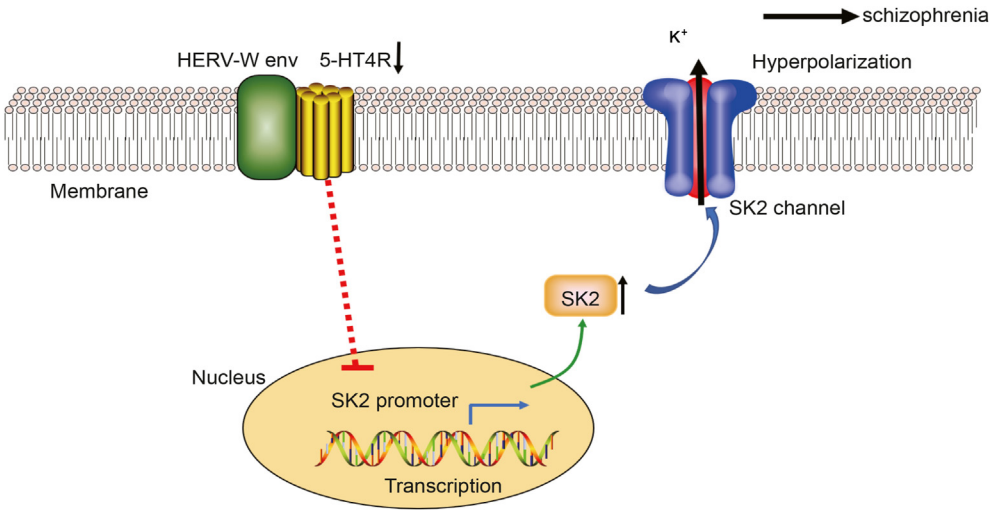

Fig. 7. A possible hypothesis is that HERV-W env may influence excitability to participate in the etiology of schizophrenia. There is a negative correlation between HERV-W env and 5-HT4R in schizophrenia. HERV-W env can directly interact with 5-HT4R and downregulate 5-HT4R expression levels. Additionally, 5-HT4R participates in the regulation of SK2 promoter activity induced by HERV-W env. Hence, there is an increased level of SK2 expression and activating SK2 channels in the cells. The opening of the SK2 channel leads to hyperpolarization and increases K^+ current, which affects the excitability of the cell membrane to participate in the development of schizophrenia.

system and have a critical role in various brain functions (Sailer et al., 2004). Dysregulation of the SK2 channel has also been implicated in neurodegenerative diseases, including Angelman syndrome and Parkinson's disease (Mourre et al., 2017; Sun et al., 2020). It is involved in alterations in synaptic plasticity and impairment in learning and memory. Evidence reveals that SK2 channels can regulate neuronal excitability in distinct compartments of the human hippocampus, which is a crucial region for memory formation (Fakira et al., 2014; Hammond et al., 2006; Willis et al., 2017). It has been reported that the blockade of SK2 channels can ameliorate learning disabilities (Mohammad et al., 2020). Learning is associated with changes in the membrane excitability of neurons. Mice knockout of SK2 shows enhanced excitability (Grasselli et al., 2020), implying that SK2 plasticity and excitability modulation are essential for cognitive symptoms of schizophrenia. Here, we found HERV-W env increased the current of the SK2 channel and activated SK2 channels. Activator of SK2 channels results in a repolarizing conductance diminishes excitatory post-synaptic potentials (EPSPs) and attenuates the spine Ca^{2+} transient (Ngo-Anh et al., 2005). Taking together, our results showed that HERV-W env might participate in the cognitive symptoms of schizophrenia via activating the SK2 channel.

Moreover, 5-HT4 receptor agonists induce long-lasting inhibition of inward K^+ currents (Eglen et al., 1995). RS67333 is a partial 5-HT4R agonist that has been widely used on cognitive effects (Lamirault and Simon, 2001). It has been reported that RS67333 could reduce K^+ currents, increase Na^+ currents and action potentials to influence neuronal excitability (Tsutsui et al., 2008). Furthermore, 5-HT4R agonists might increase neuronal excitability via decreasing the Ca^{2+}-activated-K^+ (SK) currents responsible for after-hyperpolarization (AHP) in the hippocampus (King et al., 2008; Bickmeyer et al., 2002). Electrophysiological recordings showed that HERV-W env-induced SK2 currents were suppressed when applying the 5-HT4R agonist RS67333. The significant decrease of SK2 current in the presence of RS67333 implied that 5-HT4R was a limiting factor of SK2 currents activation. A reduction of SK2 currents would enhance neuronal excitability. SK2 channels are essential for shaping postsynaptic responses and for controlling intrinsic excitability. SK2 channels internalization from the postsynaptic density (PSD) is involved in long-term potentiation (LTP) (Lin et al., 2008). Blocking SK2 channels increases the amount of LTP (Fakira et al., 2014). Hippocampal LTP impairment is a sign of cognitive symptoms of schizophrenia. These suggested HERV-W env activated SK2 channels via decreasing the 5-HT4R activity, then reduced excitability, and finally contributed to the etiology of schizophrenia.

5. Conclusions

Our clinical data suggested that there was a decreased plasma level of 5-HT4R in schizophrenia compared with healthy controls, and a negative correlation between 5-HT4R and HERV-W env in schizophrenia. *In vitro* studies showed that HERV-W env could directly interact with 5-HT4R and decrease the levels of 5-HT4R. Moreover, HERV-W env could increase SK2 expression by enhancing its promoter activity. Importantly, 5-HT4R participated in the regulation of HERV-W env-induced SK2 expression. Further studies indicated that HERV-W env activated the SK2 channel via decreased 5-HT4R. It may provide a new idea to understand the etiology of schizophrenia (Fig. 7).

Data availability

All data generated or analyzed during this study are included in this published article.

Ethics statement

The study was approved by the Medical Ethics Committee of Wuhan Mental Health Center (KY2019.02.06). Informed consent has been obtained from the legal guardian before drawing blood.

Author contributions

Xiulin Wu: investigation, data curation, methodology, and writing - original draft. Qiujin Yan: data curation and validation. Lianzhong Liu: data curation. Xing Xue: software. Wei Yao: formal analysis. Xuhang Li: formal analysis. Wenshi Li: data curation and visualization. Shuang Ding: validation. Yaru Xia: formal analysis and supervision. Dongyan Zhang: methodology. Fan Zhu: conceptualization, project administration, and writing (review and editing).

Conflict of interest

All authors declare that they have no competing interests.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2022.08.005.

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