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HERV-W env regulates calcium influx via activating TRPC3 channel together with depressing DISC1 in human neuroblastoma cells

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Abstract

The activation and involvement of human endogenous retroviruses W family envelope gene (HERV-W env, also called ERVWE1) have been reported in several neuropsychiatric disorders, including schizophrenia, as well as in multiple sclerosis (MS). Dysregulation of intracellular calcium content is also involved in the pathogenesis of these diseases. Our previous studies showed that HERV-W env overexpression results in activation of small conductance Ca^{2+}-activated K^+ channel protein 3 (SK3), a potential risk factor for schizophrenia. In the present study, we aimed to elucidate the relationship between HERV-W env and calcium signaling in schizophrenia. Our results showed that HERV-W env could induce Ca^{2+} influx in two human neuroblastoma cell lines and upregulate the expression and activation of TRPC3 in cells. The abnormal increase in intracellular Ca^{2+} concentration was inhibited by addition of the TRPC3 channel blocker pyr3, demonstrating that the Ca^{2+} influx induced by HERV-W env was TRPC3-dependent. Further experiments showed that HERV-W env overexpression downregulated DISC1, while knockdown of DISC1 promoted activation of TRPC3 without affecting TRPC3 expression. In conclusion, HERV-W env induced Ca^{2+} influx in human neuroblastoma cells by activating the TRPC3 channel through directly regulating its expression or downregulating DISC1, which could also increase TRPC3 activation without affecting TRPC3 expression. These findings provide new insights into how HERV-W env affects neuronal activity and contributes to the pathogenesis of schizophrenia.

Keywords HERV-W env · Intracellular Ca^{2+} concentration · TRPC3 · DISC1 · Schizophrenia

Introduction

Human endogenous retroviruses (HERVs), which include approximately 98,000 ERV elements and fragments constituting up to 8% of the human genome, are remnants of retroviral infections of the germline cells of our ancestors that occurred over millions of years of human evolution (Belshaw et al. 2004). To date, approximately 3000 HERV sequences have been identified (Vargiu et al. 2016), and many more are being discovered with the continuous advancement of analytical techniques (Wildschutte et al. 2016). Most HERVs contain intragenic deletions or nonsense mutations and are presumed to be silent (Griffiths 2001). Only a few retroviral genes still annotate intact open reading frames (ORFs) and encode functional proteins (Frendo et al. 2003). Although HERV antigens may normally express at low levels by the host, environmental factors, such as hypoxia (Brutting et al. 2018), drugs (Liu et al. 2013), and virus (Liu et al. 2017), in addition to mutations (Yu et al. 2014), can lead to abnormal HERV gene expression, which could then initiate or maintain pathological processes, possibly contributing to some diseases. The correlation between HERV-W and multiple sclerosis (MS) has been confirmed by reproducing the disease in transgenic mice (Antony et al. 2007), and by many other studies (Emmer et al. 2018; Morandi et al. 2017; Perron et al. 2013; Perron et al. 1997). The causative link between amyotrophic lateral sclerosis (ALS) and HERV has also been demonstrated in transgenic mice (Li et al. 2015). Schizophrenia is often associated with an inflammatory response in the central nervous system (Leza et al. 2015), and its onset is thought to be

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associated with HERVs (Huang et al. 2011; Huang et al. 2006; Qin et al. 2016; Tu et al. 2017; Wang et al. 2018; Xiao et al. 2017). Through increasing the expression of inflammatory-related genes such as inducible nitric oxide synthase (iNOS) (Xiao et al. 2017) and C-reactive protein (CRP) (Wang et al. 2018), HERVs could trigger neuroinflammatory, which may be involved in the pathogenesis of schizophrenia. Additionally, pathogenesis of various cancers are considered to be related to HERV either (Bannert et al. 2018; Cegolon et al. 2013; Grandi and Tramontano 2018; Yu et al. 2014).

The human genome harbors hundreds of elements of the HERV-W provirus. ERVWE1, a locus on chromosome 7q, contains a member of the HERV-W family with a single complete ORF that putatively encodes an envelope protein (HERV-W env, also known as Syncytin-1) (Kury et al. 2018). HERV-W env performs vital functions in the fusogenic (Frendo et al. 2003) and immunosuppressive activity in placental morphogenesis (Denner 2016). Recent studies show that aberrant expression of HERV-W env has been associated with human diseases, such as cancer (Yu et al. 2014), multiple sclerosis (Antony et al. 2007; Emmer et al. 2018; Morandi et al. 2017; Perron et al. 2013; Perron et al. 1997), and schizophrenia (Huang et al. 2011; Qin et al. 2016; Tu et al. 2017; Wang et al. 2018; Xiao et al. 2017). In our previous study, it was found that overexpression of HERV-W env resulted in increased levels of small conductance Ca^{2+}-activated K^+ channel protein 3 (SK3) in human neuroblastoma cells (Li et al. 2013). Activation of SK3, which is induced by calcium and essential for synaptic plasticity and memory, is considered a potential risk factor for schizophrenia (Grube et al. 2011).

Schizophrenia is a complex disorder associated with genetic and environmental factors. Calcium and related molecules, which take part in the development of neurons and synaptic transmission, are involved in the pathogenesis of schizophrenia (Berridge 2012). Notably, the homeostasis of intracellular calcium signaling is responsible for maintaining many neural functions. Patients with schizophrenia and other mental disorders such as Alzheimer's disease appear to have calcium imbalance (Berridge 2012). Calcium can flows into the cytoplasm through multiple channels. Calcium influx via transient receptor potential cation channel subfamily C member 3 (TRPC3) can activate extracellular signal-regulated kinases (Erk) and calcium/calmodulin-dependent protein kinase IV (CaMKIV), which in turn activates cAMP response element binding protein (CREB) signal pathway to promote neuronal survival (Jia et al. 2007; Sossin and Barker 2007). Additionally, calcium influx via TRPC3 may effect on nerve-growth-cone guidance by brain-derived neurotrophic factor (BDNF) (Li et al. 2005), which is a well-known schizophrenia-associated gene (Autry and Monteggia 2012). As a member of the store-operated calcium channel (SOCC) family, TRPC channel function can be modulated by disrupted-in-schizophrenia 1 (DISC1) (El-Hassar et al. 2014). DISC1 is involved in neurogenesis, neuronal migration, and the formation of axons, dendrites, and synapses (Brandon et al. 2009). Mutation and reduction of DISC1 protein results in predisposal to a variety of psychiatric disorders, including schizophrenia, bipolar disorder, recurrent major depression, and autism (Greenhill et al. 2015; Trossbach et al. 2014).

Although intracellular calcium concentration ($[Ca^{2+}]_i$), TRPC3 signaling, and DISC1 expression have all been found to be relevant to schizophrenia, the relationships among these factors remain unknown. In the present study, we investigated whether HERV-W env can affect the $[Ca^{2+}]_i$ in human neuroblastoma cells through flow cytometry, and studied the signaling pathways responsible for the HERV-W env–induced increase in $[Ca^{2+}]_i$ using real-time polymerase chain reaction (PCR), western blot, flow cytometric, and whole-cell patch clamp. HERV-W env overexpression in neuroblastoma cell lines resulted in increased $[Ca^{2+}]_i$ that was mediated via the TRPC3 channel. HERV-W env could activate the TRPC3 channel by directly regulating its expression or by downregulating DISC1 expression, which was shown to lead to the activation of TRPC3 without affecting the expression of TRPC3. A better understanding of the relationships among these factors might reveal the potential regulatory network underlying the functions of HERV-W env and provide greater insight into the pathogenesis of schizophrenia.

Material and methods

Cell culture and transfection

Two lines of human neuroblastoma cells, SH-SY5Y and IMR-32, were obtained from American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C with 5% CO_2. Plasmid pCMV-env was prepared as previously described (Huang et al. 2011). Transient transfection was performed using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. At 48 h post-transfection, cells were collected for further analysis.

Drug application

Pyr3 (Sigma-Aldrich, USA), a specific blocker of TRPC3 channel, was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 0.7 μM (Ma et al. 2011). During flow cytometry experiments, pyr3 was added to cells after the fluorescent dye was loaded. In patch-clamp experiments, pyr3 was added to the bath solution to block the TRPC3 current.

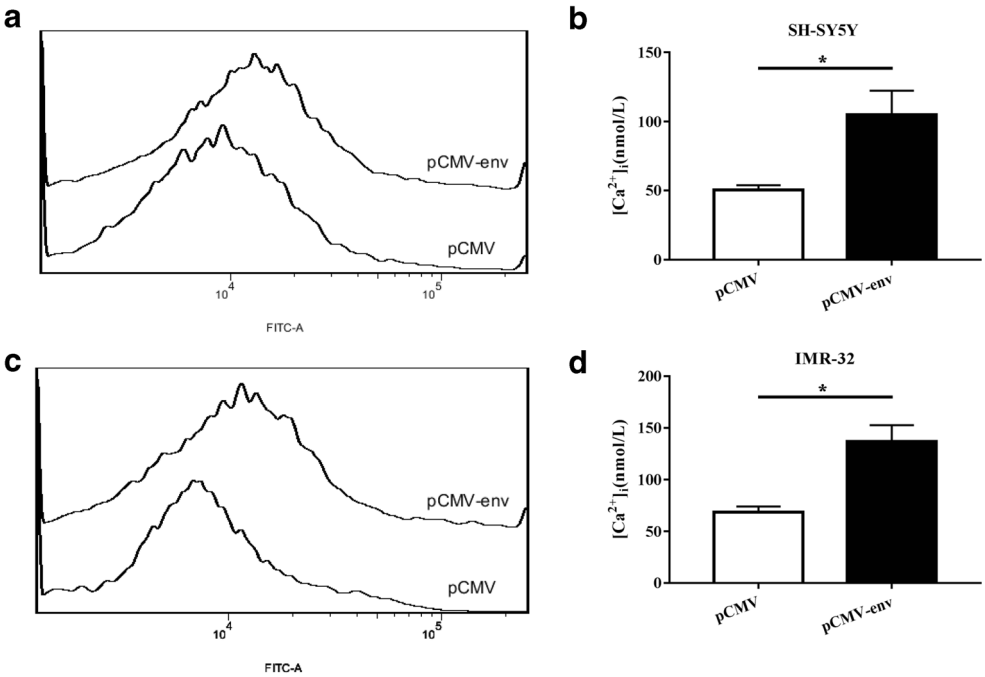

Fig. 1 HERV-W env induced Ca^{2+} influx in human neuroblastoma cells. After transfection, $[Ca^{2+}]_i$ was measured using Fluo 8-AM. $[Ca^{2+}]_i$ was increased by 108.1% in SH-SY5Y cells (**a**, **b**) and 98.8% in IMR-32 cells (**c**, **d**) transfected with pCMV-env. $^*p < 0.05$

Quantitative real-time PCR

Total RNA was isolated from cells using TRIzol Reagent (Invitrogen, USA). First-strand cDNA synthesis was performed using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, USA). Quantitative real-time PCR was performed on the Mini Opticon Detect (Bio-Rad, USA) using SYBR Select Master Mix (Life Technologies, USA). β-Actin was used as an internal control gene to normalize gene expression for quantitative comparison by the $2^{-\Delta\Delta Ct}$ method. The PCR cycling conditions were 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. Primers were designed with Primer Premier 5.0(Premier Biosoft, USA), according to the sequence of TRPC3 (NM_001130698.1) and DISC1 (NM_018662.2): TRPC3, 5′-AGAATGACTATCGGAAGCTCTCC-3′ (sense) and 5′-GGCAAGTTTGACACGACTTAATG-3′ (antisense); DISC1: 5′-ACGGCTAAAGACCTCACC-3′ (sense) and 5′-GACATTCCTGGAACTCAACA-3′ (antisense). The primers used for β-actin were as previously described (Zwart et al. 2007): 5′-CCTGGCACCCAGCACAAT-3′ (sense) and 5′-GGGCCGGACTCGTCATACT -3′ (antisense).

Western blot

Cells were lysed in M-PER reagent (Amersham, Sweden), containing protease inhibitor cocktail (Roche, Germany). Protein concentrations were determined using a BCA assay kit (Amersham, Sweden), and proteins were fractionated by 10% SDS-PAGE, transferred to PVDF membrane, followed by western blot analysis using primary antibody: anti-β-actin (1:10000; A5441, Sigma-Aldrich, USA), anti-DISC1 (1:2000; ab192258, Abcam, USA), and anti-TRPC3 (1:1000; ab188802, Abcam, USA). Membranes were washed in TBST and sequentially incubated with an HRP-conjugated anti-rabbit secondary antibody (1:5000; ab6721, Abcam, USA) for 2 h, and then bands were visualized by ECL Kit (Amersham, Sweden).

Measurement of intracellular calcium

Intracellular calcium concentration ($[Ca^{2+}]_i$) was measured using cell-permeant Ca^{2+}-sensitive fluorescent dye Fluo-8AM (AAT Bioquest, USA). The fluorescence was detected by flow cytometry with an excitation wavelength of 490 nm and an emission wavelength of 525 nm. The intracellular free calcium concentration was calculated using the following equation: $[Ca^{2+}]_i = K_d \times [F - F_{min}]/F_{max} - F]$, where F is the fluorescence of the indicator at experimental calcium levels, F_{min} is the fluorescence in the absence of calcium and F_{max} is the fluorescence of the calcium-saturated probe. The dissociation constant (K_d) of the probe is 389 nM.

RNA interference

Oligonucleotides coding short hairpin RNAs (shRNAs) were cloned into the BamHI/HindIII restriction sites of the pSilencer 2.1-U6 neo shRNA expression vector (Ambion, USA). Targeting sequences used were as follows: shDISC1 (Park et al. 2015): 5′-AAGGAAAATACTATGAAGTAC-3′; shTRPC3 (Kim et al. 2013): 5′-CCCAGTTTACATGG ACTGAAA-3′.

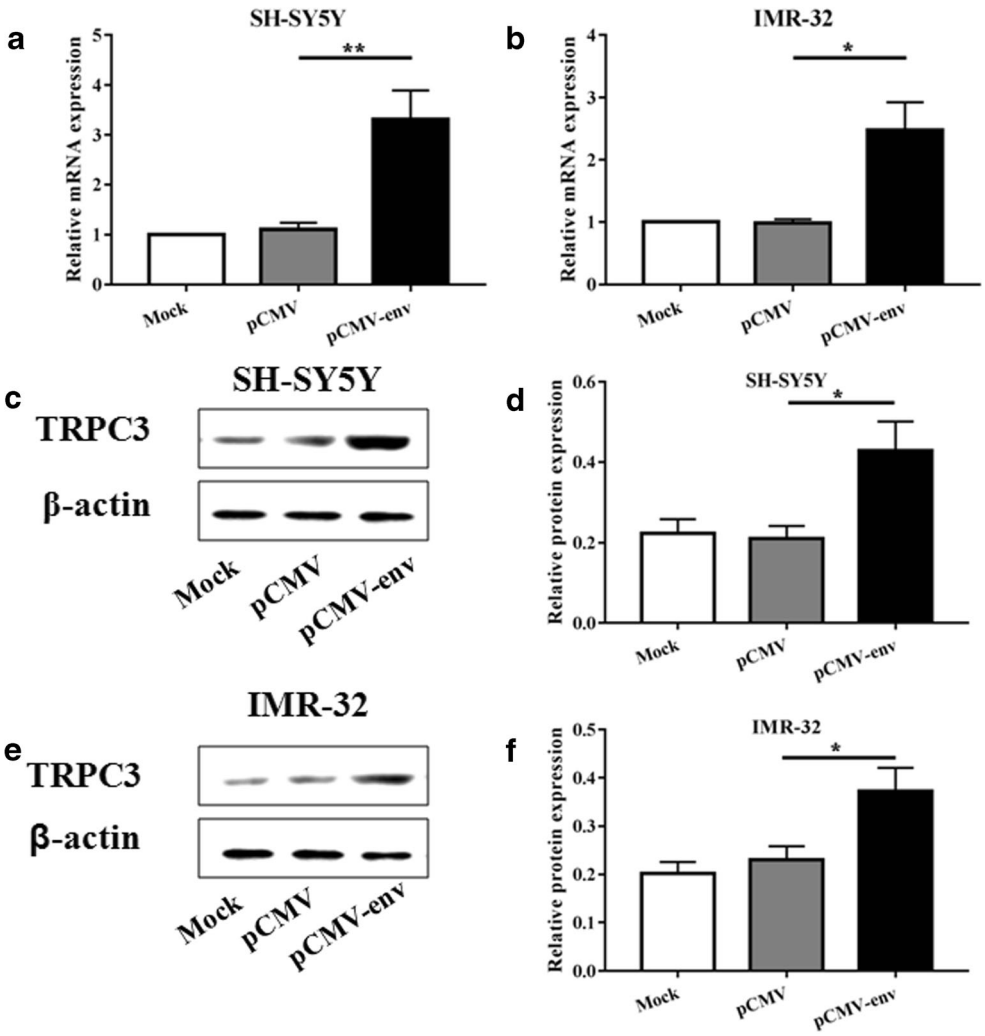

Fig. 2 HERV-W env increased expression of TRPC3. **a**, **c**, **d** In SH-SY5Y cells, HERV-W env overexpression by transfection with pCMV-env increased the mRNA expression of TRPC3 by 201.2% and the protein expression of TRPC3 by 103.8%. **b**, **e**, **f** In IMR-32 cells, HERV-W env overexpression by transfection with pCMV-env increased the mRNA expression of TRPC3 by 152.8% and the protein expression of TRPC3 by 61.3%. $*p < 0.05$, $**p < 0.01$

Electrophysiology

Cells were studied using whole-cell patch clamp. For the measurement of TRPC3 currents, the internal and external solutions were prepared according to previously reported procedures (Ma et al. 2011). Briefly, the internal solution had the following composition (in mM): CsCl 130, NaCl 5, MgATP 3, EGTA 10, and HEPES 10, pH 7.2 (adjusted with 1 M CsOH). The external solution consisted of (in mM): NaCl 130, TEA-Cl 10, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10, and glucose 10, pH 7.4 (adjusted with 1 M NaOH). After breaking the cell membrane, the following blockers (in mM) were added: nifedipine 0.002, $BaCl_2$ 0.25, tetrodotoxin (TTX) 0.01, 4-aminopyridine (4-AP) 2, and zatebradine 0.01 into the bath solution. The membrane potential was clamped at − 80 mV for 50 ms, followed by ramps from − 160 to + 60 mV for 200 ms. Between ramps, the membrane potential was held at 0 mV for 5 s. Currents were recorded using an EPC-10 patch clamp amplifier (Heka Electronic, Germany). Data were collected and initially analyzed with the Patchmaster software (Heka Electronic, Germany).

Statistical analysis

Experiments were performed in triplicate and repeated three times with similar results. Statistical analyses were performed using one-way ANOVA or Student's *t* test using GraphPad Prism 7 (Graphpad Software Inc., USA). All data were expressed as the mean ± SD, and $p < 0.05$ was considered to be statistically significant.

Results

HERV-W env induced Ca^{2+} influx in human neuroblastoma cells

Although both $[Ca^{2+}]_i$ and HERV-W env protein have been shown to be involved in schizophrenia, there is no report about the potential correlation between them. Thus, to determine the role of HERV-W env in Ca^{2+} influx, experiments were performed in the human neuroblastoma cell SH-SY5Y transfected with pCMV-env or pCMV

(Supplementary Fig. S1). The $[Ca^{2+}]_i$ was increased by 108.1% in cells transfected with pCMV-env ($p < 0.05$, Fig. 1a, b), and HERV-W env overexpression also induced a $[Ca^{2+}]_i$ increase in another human neuroblastoma cell line, IMR-32. After transfection with pCMV-env, a 98.8% increase in $[Ca^{2+}]_i$ was found in these cells ($p < 0.05$, Fig. 1c, d). These results demonstrate that overexpression of HERV-W env can induce Ca^{2+} influx in human neuroblastoma cells.

HERV-W env increased expression and triggered activation of TRPC3, which led to an increased Ca^{2+} influx

Several types of calcium channels regulate calcium influx. As one channel allowing Ca^{2+} influx into cells, TRPC3 is involved in the pathogenesis of schizophrenia (Li et al. 2005). To evaluate the effects of HERV-W env on TRPC3, mRNA and protein expressions of TRPC3 were detected after overexpression of HERV-W env in human neuroblastoma cells. The mRNA level of TRPC3 was 201.2% higher in SY-SH5Y cells transfected with pCMV-env for 48 h than in control cells ($p < 0.01$, Fig. 2a). Consistently, TRPC3 protein expression was increased by 103.8% ($p < 0.05$, Fig. 2c, d). Similar results were observed in IMR-32 cells, with HERV-W env overexpression leading to 152.8% and 61.3% increases in the mRNA and protein expression of TRPC3, respectively ($p < 0.05$, Fig. 2b, e, f).

To assess the effect of HERV-W env on TRPC3 channel function, we performed whole-cell patch clamp experiments in SY-SH5Y cells overexpressing HERV-W env. As shown in Fig. 3, the current was increased in HERV-W env-overexpressing cells compared to that in control cells, with the average current density (38.3 ± 6.0pA/pF) enhanced by 55.7% ($p < 0.05$). After treatment with pyr3, the average current density dropped to 23.1 ± 5.1pA/pF, which decreased by 39.7% ($p < 0.05$, Fig. 3a, b), and $[Ca^{2+}]_i$ were decreased to 47.2% ($p < 0.05$, Fig. 4a, b). The expression of TRPC3 was knocked down by shTRPC3 (Supplementary Fig. S2), and $[Ca^{2+}]_i$ was then decreased by 58.7% ($p < 0.01$) in HERV-W env-overexpressed SH-SY5Y cells (Fig. 5a, b).

Similarly, in IMR-32 cells, the average current density (35.2 ± 4.0 pA/pF) was enhanced by 49.2% in HERV-W env-overexpressing cells ($p < 0.05$, Fig. 3c, d), whereas pyr3 treatment blocked the enhancement of Ca^{2+} current caused by HERV-W

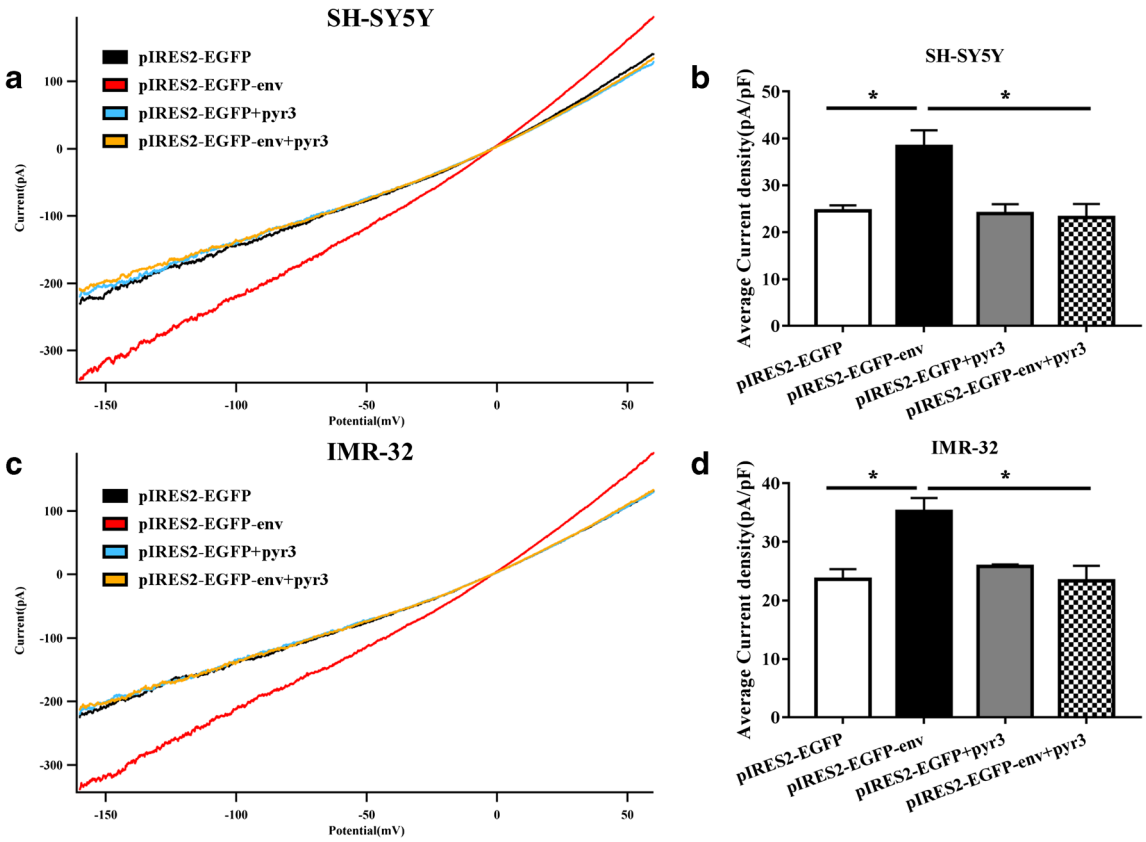

Fig. 3 HERV-W env triggered activation of TRPC3 channel. **a**, **c** TRPC3 currents in human neuroblastoma cells detected by whole-cell patch-clamp before and after addition of pyr3 (0.7 μM) to the external solution in **a** SH-SY5Y cells and **c** IMR-32 cells. **b**, **d** Histograms showing the average TRPC3 current amplitudes in human neuroblastoma cells, expressed as current density in pA/pF. Notably, the TRPC3 current was increased by 55.7% in SH-SY5Y cells (**b**) and 49.2% in IMR-32 cells (**d**) compared with that in control cells. *$p < 0.05$

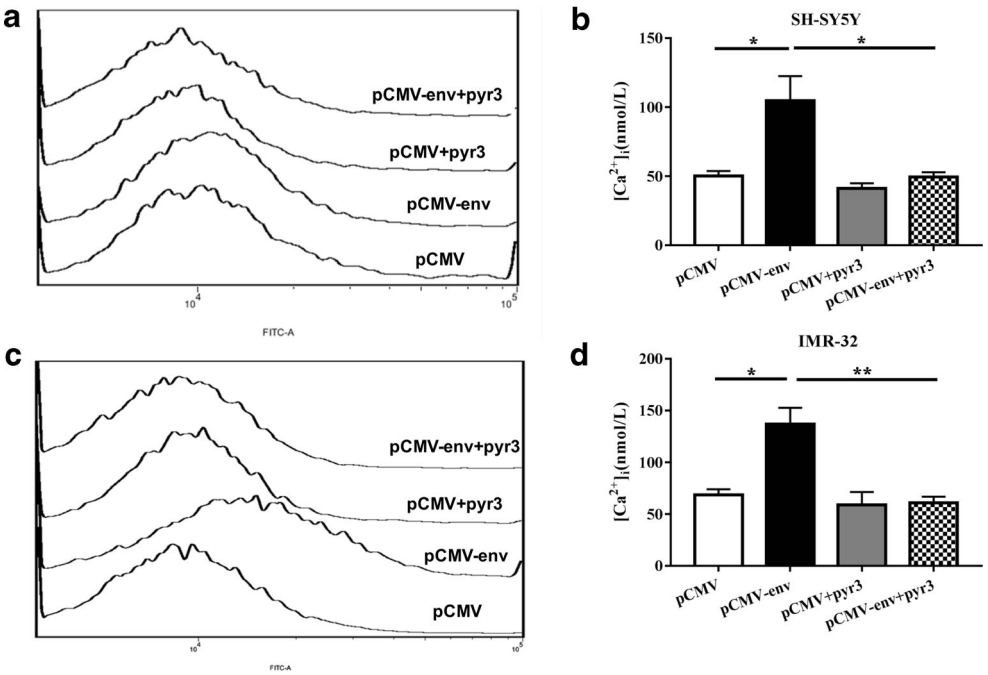

Fig. 4 The HERV-W env–induced increase in Ca^{2+} influx was dependent on TRPC3. In the presence of pyr3, the $[Ca^{2+}]_i$ was decreased to 47.2% and 44.5% in HERV-W env-overexpressing SH-SY5Y (**a**, **b**) and IMR-32 (**c**, **d**) neuroblastoma cells, respectively. $*p < 0.05$, $**p < 0.01$

env: 33.8% decrease in average current density ($p < 0.05$, Fig. 3c), and 55.5% decrease in $[Ca^{2+}]_i$ ($p < 0.01$, Fig. 4c, d). Given the substantial variability, we observed a decline of $[Ca^{2+}]_i$ (56.8%) in HERV-W env–overexpressed cells after knockdown of TRPC3 ($p < 0.01$, Fig. 5c, d). These data suggest that overexpression of HERV-W env may lead to upregulated expression and increased activation of TRPC3.

HERV-W env downregulated the expression of DISC1

In our previous studies, we found that HERV-W env could induce the expressions of some schizophrenia-associated genes, such as BDNF, dopamine receptor D3 (DRD3), SK3, and others. DISC1, a promising schizophrenia

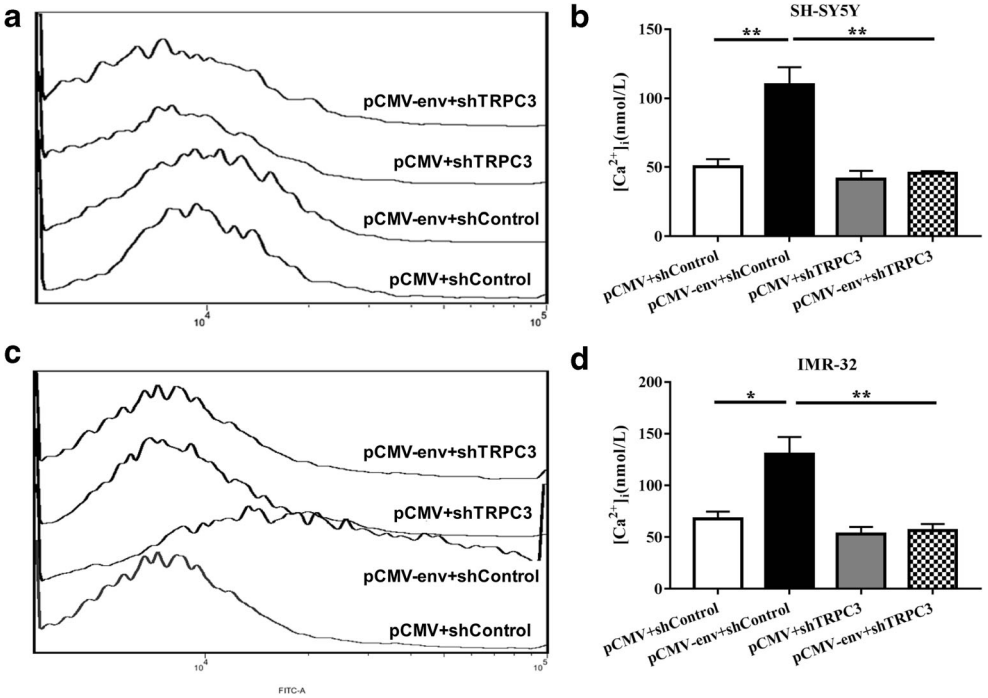

Fig. 5 HERV-W env increased Ca^{2+} influx through TRPC3. Knockdown of TRPC3 using shTRPC3 resulted in 58.7% and 56.8% decreases in $[Ca^{2+}]_i$ in HERV-W env–overexpressing SH-SY5Y and IMR-32 cells, respectively. $*p < 0.05$, $**p < 0.01$

candidate gene, also involves in the pathogenesis of schizophrenia. However, there has been no report on the potential relationship between HERV-W env and DISC1. In the present study, we found that DISC1 mRNA expression was significantly lower in HERV-W env–overexpressing SH-SY5Y cells than in control transfected cells. After 48 h of transfection, DISC1 mRNA decreased to 53.6% ($p < 0.01$, Fig. 6a). Consistently, subsequent western blot experiments showed that DISC1 protein expression was decreased to 41.3% ($p < 0.01$, Fig. 6c, d).

In IMR-32 cells, DISC1 expression was also found to decrease after transfection with pCMV-env. Real-time PCR showed a 51.5% decrease in the DISC1 mRNA level ($p < 0.01$, Fig. 6b), and western blot analysis indicated a 65.5% decrease in the DISC1 protein level in pCMV-env-transfected cells ($p < 0.01$, Fig. 6e, f). These results suggest that HERV-W env decreased DISC1 expression in neuroblastoma cells.

Downregulation of DISC1 contributed to Ca^{2+} influx through TRPC3

The decreased DISC1 expression is known to affect calcium dynamics (Park et al. 2015). Therefore, shDISC1 was used to knockdown the expression of DISC1 (Supplementary Fig. S3), and consequently, $[Ca^{2+}]_i$ was increased by 33.6% ($p < 0.05$) in SH-SY5Y cells transfected with shDISC1, demonstrating that the downregulation of DISC1 could increase $[Ca^{2+}]_i$ (Fig. 7a, b). $[Ca^{2+}]_i$ was also increased in IMR-32 cells after knockdown of DISC1 ($p < 0.05$, Fig. 7c, d).

The TRPC3 channel blocker pyr3 was used to assess the role of TRPC3 in DISC1-mediated Ca^{2+} dysregulation after knockdown of DISC1. Remarkably, with pyr3 treatment, $[Ca^{2+}]_i$ was decreased by 32.2% ($p < 0.05$) and 42.5% ($p < 0.05$) in HERV-W env–overexpressing SH-SY5Y and IMR-32 cells, respectively (Fig. 7). Similarly, 32.6%

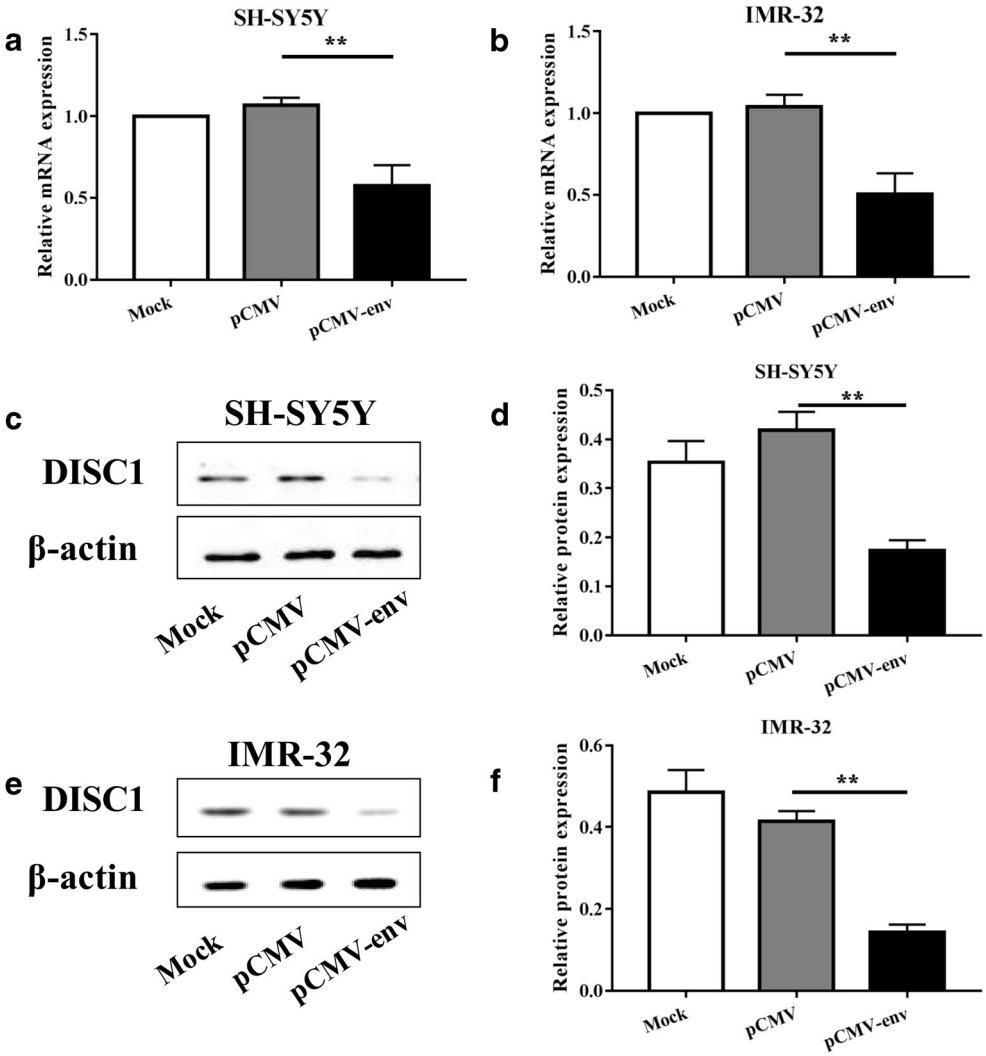

Fig. 6 HERV-W env overexpression downregulated DISC1 expression. **a**, **c**, **d** In SH-SY5Y cells, HERV-W env overexpression decreased the DISC1 mRNA expression to 53.6% and the DISC1 protein expression to 51.5%. **b**, **e**, **f** In IMR-32 cells, HERV-W env overexpression decreased the DISC1 mRNA expression to 65.5% and the DISC1 protein expression to 34.5%. $^{**}p < 0.01$

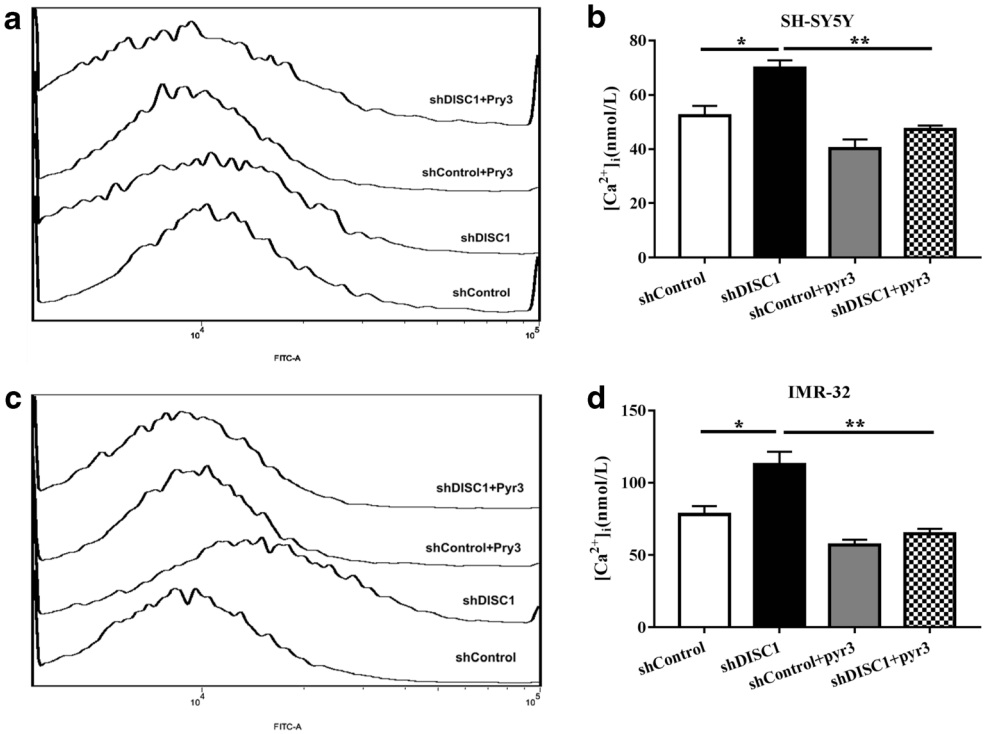

Fig. 7 Reduced DISC1 expression contributes to Ca^{2+} influx through the TRPC3 channel. **a**, **b** $[Ca^{2+}]_i$ was increased by 33.6% in SH-SY5Y cells transfected with shDISC1, demonstrating that knockdown of DISC1 could increase $[Ca^{2+}]_i$. **c**, **d** A 44.0% increase in $[Ca^{2+}]_i$ was observed in IMR-32 cells with knockdown of DISC1. With pyr3 treatment, the marked increase in $[Ca^{2+}]_i$ induced by DISC1 knockdown was abolished. $^*p < 0.05$, $^{**}p < 0.01$

($p < 0.05$) and 47.0% ($p < 0.05$) decreases in $[Ca^{2+}]_i$ were observed in cells with knockdown of TRPC3, using shTRPC3 (Fig. 8). These results indicate that the TRPC3 channel played a pivotal role in the abnormal elevation of $[Ca^{2+}]_i$ caused by decreased DISC1.

Downregulation of DISC1 activated TRPC3 channel, without affecting its expression

We next studied the effects of DISC1 on the TRPC3 expression by real-time PCR and western blot. The mRNA ($p =$

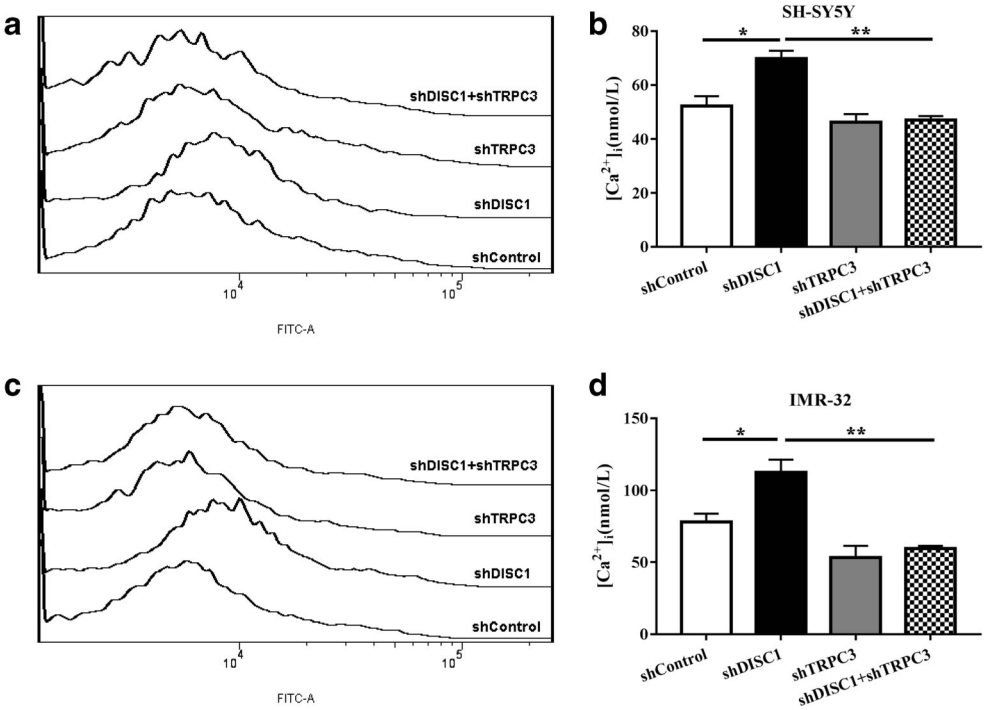

Fig. 8 Downregulation of TRPC3 could inhibit the $[Ca^{2+}]_i$ influx induced by knockdown of DISC1. In HERV-W env–overexpressing SH-SY5Y and IMR-32 cells, 32.6% (**a**, **b**) and 47.0% (**c**, **d**) decreases in $[Ca^{2+}]_i$, respectively, were observed with knockdown of TRPC3. $^*p < 0.05$, $^{**}p < 0.01$

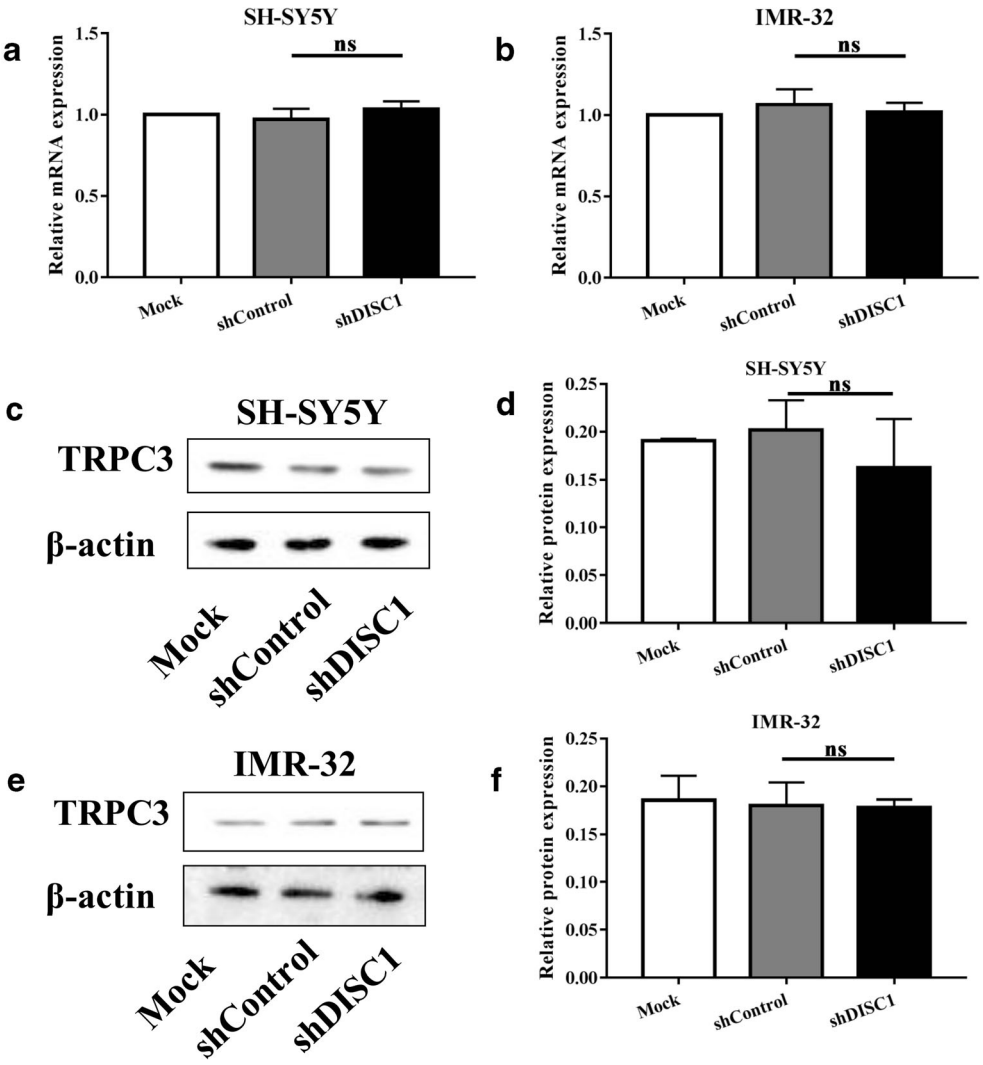

Fig. 9 Knockdown of DISC1 did not affect the expression of TRPC3. After transfection with shDISC1, the mRNA and protein levels of TRPC3 were detected in neuroblastoma cells. No significant differences in the mRNA (**a** SH-SY5Y cells; **b** IMR-32 cells) and protein (**c**, **d** SH-SY5Y cells; **e**, **f** IMR-32 cells) levels were observed between cells transfected with shDISC1 and control cells

0.868) or protein ($p = 0.726$) expression levels of TRPC3 did not differ between SH-SY5Y cells transfected with shDISC1 for 48 h and control cells (Fig. 9a, c, d). Similar results were also obtained in IMR-32 cells, which showed little variation in the mRNA ($p = 0.646$) or protein ($p = 0.997$) levels of TRPC3 after knockdown of DISC1 (Fig. 9b, e, f).

To assess the effect of DISC1 on TRPC3 channel activation, we performed whole-cell patch clamp experiments in SY-SH5Y and IMR32 cells. Shown in Fig. 10, there were notable 20.8% ($p < 0.05$) and 19.4% ($p < 0.05$) increases in the average current density in SY-SH5Y and IMR32 cells transfected with shDISC1, respectively, compared with those in control cells, and the average current density decreased by 23.3% ($p < 0.05$) and 21.9% ($p < 0.05$) in the respective cell types upon treatment with the TRPC3 channel blocker pyr3. These results indicate that reduced DISC1 expression led to activation of the TRPC3 channel rather than a change in its mRNA or protein expression.

In summary, our data indicate that overexpression of HERV-W env induced Ca^{2+} influx through TRPC3 channel in two human neuroblastoma cell lines. HERV-W env could activate TRPC3 channel via upregulating its expression or silencing DISC1 expression, while downregulation of DISC1 did not affect TRPC3 expression.

Discussion

HERVs were long-misunderstood to be junk DNA, but some are now considered to have critical physiological functions (Kowalski and Mager 1998; Mangeney et al. 2001; Mi et al. 2000). Triggered by variable environmental factors (Brutting et al. 2018; Liu et al. 2013; Liu et al. 2017; Yu et al. 2014), the aberrant expression of HERVs relates to a number of diseases, such as multiple sclerosis (Antony et al. 2007; Emmer et al. 2018; Morandi et al. 2017; Perron et al. 2013; Perron et al.

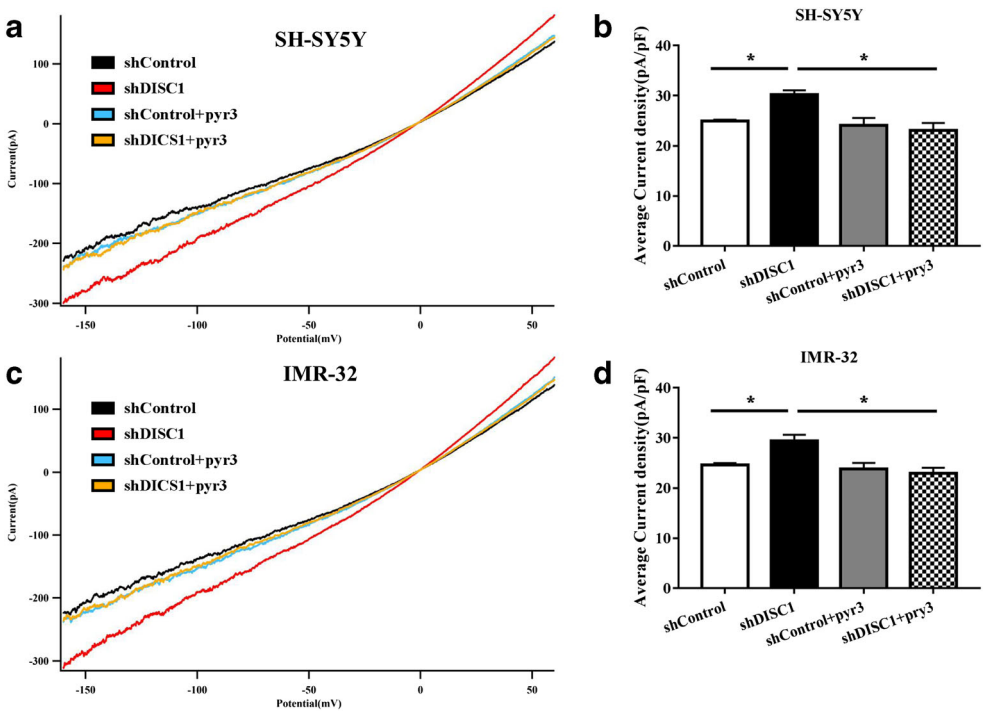

Fig. 10 Knockdown of DISC1 activated the TRPC3 channel. **a**, **c** TRPC3 currents in human neuroblastoma cells were detected by whole-cell patch-clamp before and after addition of pyr3 (0.7 μM) to the external solution (**a** SH-SY5Y cells; **c** IMR-32 cells). **b**, **d** Histograms indicating average TRPC3 current amplitudes in human neuroblastoma cells, expressed as current density in pA/pF. Notably, with DISC1 knockdown, the TRCP3 current was increased by 20.8% in SH-SY5Y cells (**b**) and 19.4% in IMR-32 cells (**d**) compared with the currents in the respective control cells. $*p < 0.05$

1997), schizophrenia (Huang et al. 2011; Huang et al. 2006; Qin et al. 2016; Tu et al. 2017; Wang et al. 2018; Xiao et al. 2017), type 1 diabetes (Levet et al. 2017), and cancers (Bannert et al. 2018; Cegolon et al. 2013; Grandi and Tramontano 2018; Yu et al. 2014). How HREVs contribute to these illnesses has remained unclear. Some HERVs are able to code for all viral proteins and produce virus-like particles (Contreras-Galindo et al. 2015). Some of them may induce T lymphocyte response (Perron et al. 2001) and have potential relevance to diseases (Perron et al. 1997). With inserting into locus near cellular genes, HERVs could affect the expression or function of those genes, which might cause diseases (Wildschutte et al. 2016). The abnormal expression of HERV elements could also take part in the progress of diseases, through multiple pathways and regulatory factors, such as interferon gamma (IFNγ) (Manghera et al. 2015). Abnormal activation of HERV-W env may have functional roles in multiple processes such as inflammation, which may lead to disease (Kury et al. 2018). Our previous studies have shown that HERV-W env could regulate risk genes for schizophrenia such as BDNF, DRD3 (Huang et al. 2011), and SK3 (Li et al. 2013) through CREB. Another study found that glycogen synthase kinase 3β (GSK3β) might be involved in the expression of BDNF induced by HERV-W env, via the phosphorylation at Ser9 (Qin et al. 2016). HERV-W env could also promote the generation of specific cytotoxic T lymphocytes (CTLs), which pose a high killing potential toward nerve cells (Tu et al. 2017). In microglia, HERV-W env increased the production of a neuroinflammatory related factor, NO, and led to microglial migration (Xiao et al. 2017). In astrocytes and microglia, HERV-W env could induce the activation of CRP through TLR3-IL6 signaling pathway, resulting in neuroinflammation which might promote the development of schizophrenia (Wang et al. 2018). Additionally, HERV-W env was suggested to activate innate immunity through CD14/TLR4 (Rolland et al. 2006). Together, these previous findings suggest that by regulating the expression of risk genes or contributing to immune and inflammatory processes, HERV-W env may play roles in the progression of diseases such as schizophrenia.

Schizophrenia is a severe, debilitating psychiatric disorder that affects approximately 1% of the population worldwide (Oresic et al. 2011). Both genetic and environmental factors

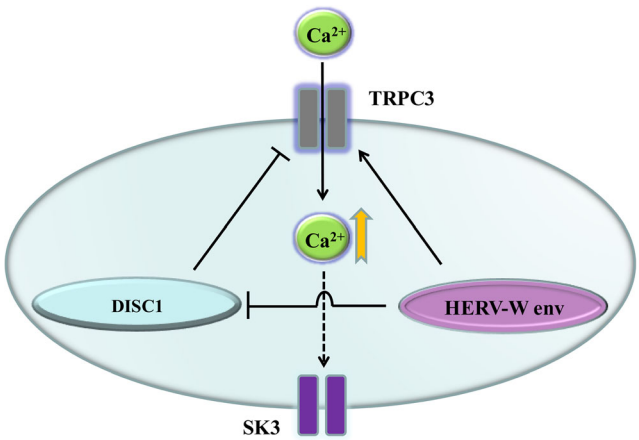

Fig. 11 HERV-W env induced calcium influx in human neuroblastoma cells. This effect was mediated via the SOCC channel TRPC3. Downregulation of DISC1 may be involved in the process

are involved, and schizophrenia is most commonly considered a neurodevelopmental disorder. Calcium and calcium channels, which play vital roles in the development of neurons and synaptic transmission, are involved in the pathogenesis of schizophrenia (Berridge 2012). Genetic analyses of large patient cohorts have found several candidate risk genes associated with schizophrenia, including genes encode proteins involved in calcium signaling, such as voltage-gated calcium channel (VGCC) subunits (Purcell et al. 2014; Samocha et al. 2014). In cortical neurons, Ca^{2+} influx via L-type VGCCs (CACNA1C) could contribute to the phosphorylation and activation of CREB, and thereby support neuronal survival (Dolmetsch et al. 2001). Our previous study demonstrated that SK3 could be regulated by HERV-W env through the activation of CREB (Li et al. 2013) and SK3 channel is calcium-dependent. We also found that NO could induce the release of Ca^{2+} in neurons (Kakizawa et al. 2013) and that HERV-W env could increase NO production (Xiao et al. 2017). In the present paper, $[Ca^{2+}]_i$ was detected in human neuroblastoma cells overexpressing HERV-W env, and an enhanced Ca^{2+} entry occurred (Fig. 1). To sum up, our results indicate that HERV-W env and $[Ca^{2+}]_i$ might play potential roles in schizophrenia.

Calcium influx via multiple channels, such as VGCCs, receptor-operated calcium channels (ROCCs) and SOCCs. TRPC channels, one subfamily of SOCCs, have been suggested to play specific roles in the developing brain (Strubing et al. 2003). As a widely expressed member of the TRPC family in the brain, the TRPC3 channel is involved in BDNF-induced axon guidance (Li et al. 2005), neuronal survival (Jia et al. 2007), and many other processes (Hartmann et al. 2008; Hartmann et al. 2011; Nelson and Glitsch 2012). In the present study, HERV-W env increased the expression of TRPC3, activated TRPC3 channel (Figs. 2 and 3), and induced Ca^{2+} influx through TRPC3 (Figs. 4 and 5). Considering that TRPC3 has been related to some mental disorder such as schizophrenia (Li et al. 2005), we hypothesize that HERV-W env may play a role in the pathogenesis of schizophrenia by inducing aberrant TRPC3 channel function. Further research to investigate this hypothesis is needed.

Depletion of calcium can be regulated by another well-known schizophrenia-associated gene DISC1, while recruitment of DISC1 to IP3R can have an impact on IP3R1 activity, leading to inhibition of calcium release from endoplasmic reticulum (ER) (Park et al. 2015). As a member of SOCCs, TRPCs can be activated by STIM1, which requires depletion of calcium from ER stores (Yuan et al. 2007). Hence, it is plausible that DISC1 deficiency can induce abnormal ER calcium dynamics (Park et al. 2015), which in turn activates TRPC3 through clustering of STIM1 (Yuan et al. 2007). The results of the present study demonstrated that reduced DISC1 could activate the TRPC3 channel (Fig. 10), without influencing its expression (Fig. 9). Induced Ca^{2+} entry through TRPC3 was also observed in DISC1 cells upon DISC1 knockdown (Figs. 7 and 8). Moreover, overexpression of HERV-W env led to significant downregulation of DISC1 (Fig. 6), suggesting that HERV-W env might play a key role in DISC1- and TRPC3-related calcium dynamics.

Notably, the present study was conducted in neuroblastoma cell lines, which do not behave as normal neurons, so it might affect the studied parameters. And animal models are also needed. The present results obtained in cell lines can still provide useful insight into how HERV sequences modulate neuronal activity and potentially influence the development of schizophrenia.

In summary, overexpression of HERV-W env may induce Ca^{2+} influx in human neuroblastoma cells via activating TRPC3 channel in two pathways: upregulation of the expression of TRPC3 or downregulation of DISC1, which does not affect the expression of TRPC3 but promotes its activation (Fig. 11). Therefore, our finding provides a potential mechanism for the relationship between HERV-W env and the $[Ca^{2+}]_i$ signaling cascade and may provide a new idea to understand the pathogenesis of schizophrenia.

Author contributions Mr. Chen conducted the overall study and drafted the manuscript; Miss Yan performed the cytological experiments; Mr. Zhou conducted data analysis; Dr. Li carried out electrophysiology experiments; Dr. Zhu served on the scientific advisory board for the School of Medicine, Wuhan University, conceived and designed the experiments, drafted the manuscript, and paid bills for this procedure.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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