A 3'UTR-Associated RNA, *FLJ11812* Maintains Stemness of Human Embryonic Stem Cells by Targeting *miR-4459*

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The 3' untranslated region (UTR)-associated RNAs (uaRNAs) have important roles in various biological processes, especially in development. However, since they overlap with protein-coding mRNAs, uaRNAs are difficult to study by RNA interference techniques. We recently identified a chemical molecule, 3-benzyl-5-((2-nitrophenoxy) methyl)-dihydrofuran-2(3H)-one (3BDO), that could efficiently induce human embryonic stem cells (hESCs) differentiation, and meanwhile selectively and efficiently downregulate the uaRNA *FLJ11812*. By acting as a competing endogenous RNA, downregulated *FLJ11812* by 3BDO further increased *miR-4459* level in hESCs. *miR-4459* could decrease the expression of its targets, *CDC20B* and *ATG13*, and thus altered stemness via cell cycle and autophagy. Our results revealed that *FLJ11812* played a key role in maintenance of stemness of hESCs for the first time. The findings provide new clues and a powerful tool for investigating the action mechanism of *FLJ11812* in early development.

Introduction

IN EUKARYOTIC EVOLUTION, the 3' untranslated regions (3'UTRs) of mRNAs underwent rapid expansion, for more sophisticated functions [1]. The regulatory elements located in the 3'UTR are involved in post-transcriptional regulation of gene expression by regulating mRNA translation, turnover, transport, stability, and subcellular localization [2,3]. 3'UTR-associated RNAs (uaRNAs) have a number of independent functions. They are involved in developmental and tissue- or cell-specific regulation and act as decoys to regulate trans-acting factors [1,4].

Many uaRNAs have been identified, but the cellular functions of most remain to be determined [1]. Due to the unique position of uaRNAs in the transcript—overlapping protein-coding mRNAs—designing specific siRNA sequences for their knockdown or knockout is difficult. In some cases, only the candidates that could be knocked down by using RNA interference (RNAi) were selected for further study [5]. Therefore, the functions of some important RNAs are not known.

To address this question, we identified a small molecule, 3-benzyl-5-((2-nitrophenoxy) methyl)-dihydrofuran-2(3H)-one (3BDO), that could selectively and efficiently downregulate the uaRNA *FLJ11812*, derived from the 3'UTR of transforming growth factor, beta 2 (*TGFB2*) [6]. *Homo sa-*

piens cDNA *FLJ11812* has been completely sequenced and characterized and was found expressed in whole embryos, mainly in the head [7]. In our previous work, we found that 3BDO activated a serine/threonine kinase:mechanistic target of rapamycin (MTOR) by targeting FKBP1A and further phosphorylated TIA1, which was responsible for the processing of *FLJ11812* by interacting with *TGFB2*. TIA1 phosphorylation reduced this interaction, and then selectively and efficiently downregulated *FLJ11812* in human umbilical vein endothelial cells (HUVECs). Furthermore, *FLJ11812* acted as a competing endogenous RNA (ceRNA) that regulated *miR-4459* and the expression of its target gene-*ATG13* thus regulated autophagy level in HUVECs [6].

In previous reports, a ceRNA, linc-RoR, was found to regulate self-renewal and differentiation of human embryonic stem cells (hESCs) [8]. ESCs, derived from the inner cell mass of blastocysts, are established models for investigating embryonic development and cell differentiation, especially in humans [9,10]. The uaRNAs can be independently expressed as developmentally regulated non-coding RNAs (ncRNAs) and may participate in regulating differentiation and developmental processes [1]. However, the functions and molecular mechanisms of *FLJ11812* during embryonic development and maintenance of hESCs stemness have not been elucidated. In this study, we used hESCs

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as models to study the role of *FLJ11812* in maintaining the stemness and differentiation of hESCs by using 3BDO to selectively inhibit *FLJ11812*.

Materials and Methods

Cell culture

hESC (H1 cell line) was obtained from Prof. Jiaxi Zhou (Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China) and maintained as routine under a feeder-independent culture system as described [11]. Experiments involved H1 cells between passages 40 and 60. HEK293 cells were grown in Dulbecco's modified Eagle's medium (Gibco) with 10% fetal bovine serum (Gibco), penicillin (50 U/mL), and streptomycin (50 µg/mL) (Gibco; 10378-016).

Fluorescent in situ hybridization

FLJ11812 expression in hESCs was detected by use of a FISH kit (Roche Applied Science). Briefly, hESCs treated with or without 3BDO were fixed in 4% paraformaldehyde, then prehybridized with hybridization solution, and incubated with a digoxigenin-labeled *FLJ11812* probe. Cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) for 5 min at room temperature. Fluorescence images were obtained by use of an LSM 700 Confocal Laser Scanning Microscope (Carl Zeiss).

Quantitative real-time polymerase chain reaction

Total RNA was extracted from ESCs by the Trizol reagent method (Invitrogen) and underwent quantitative reverse transcription PCR (RT-PCR) (Roche; Light Cycler 2.0 system) with the primer pair sequences for genes (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/scd). The reverse transcription step involved use of the PrimeScript RT reagent kit with gDNA Eraser (DRR047; Takara). Quantitative RT-PCRs involved use of SYBR Premix Ex Taq (Tli RNaseH Plus) and were carried out in a 20-µL volume with 10 µL of 2× SYBR Green I, 0.4 µL sense primer, 0.4 µL antisense primer, 2 µL cDNA template, and 7.2 µL distilled water. Relative gene expression was normalized to that of a housekeeping gene (GAPDH). The levels of expressed genes were measured by the $2^{-\Delta\Delta C_t}$ method with MxPro 4.00 (Stratagene).

Western blot analysis

Treated hESCs were lysed in protein lysis buffer (Beyotime; P0013), and protein content was determined by use of the BCA Protein Assay Kit (Beyotime; P0011). Proteins were separated by 15% or 12% SDS-PAGE and transferred to PVDF membrane (Millipore; IPVH00010), which was incubated with primary antibodies (1:1,000) for octamer-binding transcription factor 4 (Oct4; Abcam, ab18976), Nanog (Abcam; ab80892), and sex determining region Y (SRY)-box 2 (Sox2; Abcam, ab97959); ATG13 (Sigma; SAB4200100); CDC20B (Santa Cruz Biotechnology; sc-137387), and ACTB (Santa Cruz Biotechnology; sc-47778), or LC3B (Cell Signaling Technology; 2775) at 4°C overnight and detected with corresponding horseradish peroxidase (HRP)-conjugated secondary antibody (1:10,000) at room temperature for 1 h. The membrane was incubated with Immobilon Western Chemi-

luminescent HRP Substrate (Millipore; WBKLS0500) for 5 min at room temperature and exposed to X-ray film (Kodak). The relative protein content was analyzed by use of ImageJ software and normalized to loading controls.

Cell cycle analysis

hESCs were treated with or without 3BDO for 72 h, then trypsinized by adding 0.05% Trypsin/EDTA solution at 37°C for 10 min. Cells were fixed overnight with ice-cold 70% ethanol and stained with Guava Cell Cycle Reagent (Millipore; 4500-0220) at room temperature for 30 min in the dark, then analyzed by use of the Guava EasyCyte Mini flow cytometry system (Millipore). A minimum of 5,000 events was counted.

Immunofluorescence assay

Treated hESCs were fixed with 4% paraformaldehyde for 15 min, blocked with 5% normal goat serum for 20 min at room temperature, and incubated with primary antibodies (1:100) for Oct4 (Abcam; ab18976), Nanog (Abcam; ab80892), or Sox2 (Abcam; ab97959); or LC3B (Cell Signaling Technology; 2775) at 4°C overnight, then corresponding Alexa 488-labeled secondary antibody (1:200) at 37°C for 1 h. Negative control (NC) cells were incubated with normal IgG. Cells were examined under a Leica TCS SP2 confocal laser scanning microscope (Leica).

Quantitative real-time PCR for miRNA

Total RNA was isolated by the Trizol reagent method (Invitrogen). RNA content was determined by use of the NanoDrop (Thermo). *miR-4459* was converted into cDNA with use of the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems; PN4366596) and a specific stem-loop primer, 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCTGCACTG GATACGACTCCACCT-3'. The expression of mature *miR-4459* in hESCs was quantified by use of the KAPA SYBR FAST qPCR Kit (Kapa Biosystems; KK4601). Quantitative real-time PCR involved the LightCycler 480 Real-Time PCR System (Roche). Small RNA *U6* was used as an internal control for small RNAs. Primer sequences were for *miR-4459*, forward, 5'-ATTATCCAGGAGGCGGAGG-3', and reverse, 5'-TCGT ATCCAGTGCAGGGTC-3'; and *U6*, forward, 5'-CTCGCTT C GGCAGCACATATACT-3', and reverse, 5'-ACGCTTCAC GAATTTGCGTGTC-3'. *miR-4459* expression was calculated relative to *U6* expression, by three independent experiments.

RNA mimics transfection

HEK293 cells or hESCs were grown on six-well plates. The mimics of *miR-4459* was designed to mimic endogenous mature *miR-4459*. RNA mimics for *miR-4459* and NC were designed and purchased from Invitrogen and transfected into HEK293 or hESCs with use of Lipofectamine RNAiMAX Transfection Reagent (Invitrogen; 13778150) for 48 h. The final concentrations of the mimics and NC were 10 or 50 nM.

Luciferase reporter transfection and dual luciferase activity assay

HEK293 cells were seeded into 96-well plates at 6,000 cells per well for incubation overnight, then cotransfected with plasmids of dual-luciferase (containing firefly and Renilla luciferase) reporters for *CDC20B* 3'UTR (Luc-*CDC20B*

3'UTR) and *miR-4459* mimics or NC (final concentration, 10 or 50 nM) with Lipofectamine 2000 (Invitrogen; 11668-019) for 24 h. Dual luciferase activity was measured by use of the Dual-Glo Luciferase Assay System (Promega; E2920). After reagent was added, firefly luciferase or Renilla luciferase activity was measured by use of a VICTOR X2 Multilabel Plate Reader (PerkinElmer). Firefly luciferase activity was normalized to that of Renilla.

Statistical analysis

The number of LC3B puncta was acquired by using ImageJ 1.47 (US National Institutes of Health). Data are presented as mean $\pm$ SEM, and analysis involved use of SPSS 20.0.0 (SPSS, Inc.). Images were processed by use of Adobe Photoshop CS5 (Adobe). $P < 0.05$ was considered statistically significant.

Results

3BDO could efficiently induce hESCs differentiation into three layers

The chemical small molecule, 3BDO, that inhibited autophagy in HUVECs and neuronal cells [12,13], but its function in hESCs was unknown. After 3BDO treatment, we observed the morphological changes of hESCs, whose loss of ESC morphologic features presented a differentiated state in maintenance medium for hESCs (Fig. 1A). We wondered whether 3BDO influence the maintenance of hESCs stemness. The mRNA levels of core transcript factors Oct4, Sox2, and Nanog were significantly decreased in hESCs with 3BDO treatment (Fig. 1B). Their protein levels were decreased with 30 or 60 μ M 3BDO for 72 h (Figs. 1C and 2). Furthermore, we analyzed the cell cycle of hESCs with or without 3BDO treatment, and found that 3BDO-treated hESCs lost the ESC-specific cell cycle (short G1 phase) (Fig. 3A), which is crucial for maintenance of ESCs stemness [14]. Therefore, 3BDO could efficiently induce the differentiation of hESCs.

To understand which germ layer differentiation was induced by 3BDO in hESCs, we examined the expression of markers for three germ layers [Nestin, Zic1 and Sox1 for ectoderm; Nkx2.5, Brachyury (T gene) and Flk1 for mesoderm; Gata6, Gata2, Foxa2, and Sox17 for endoderm; and SSEA4 for self-renewal] in hESCs cultured with or without 3BDO. The mRNA levels of markers for the three germ layers were upregulated with 3BDO treatment (Fig. 3B). Collectively, our data showed that 3BDO could rapidly lead hESCs to lose the stemness and the ESC-specific cell cycle, and drive it to differentiate into three layers.

FLJ11812 RNA level was selectively decreased with 3BDO treatment in hESCs

In our previous work, we found that 3BDO selectively downregulated *FLJ11812* in HUVECs [6]. To understand how 3BDO promote hESCs differentiation, we detected the RNA level of *FLJ11812* in hESCs with or without 3BDO treatment. The result showed that the RNA level of *FLJ11812* was significantly decreased by 3BDO at 30 or 60 μ M for 72 h, but mRNA level of *TGFB2* was not affected (Fig. 4A). On fluorescent in situ hybridization, 3BDO at 30 or 60 μ M for 72 h significantly inhibited the expression of

FIG. 1. 3-Benzyl-5-((2-nitrophenoxy) methyl)-dihydrofuran-2(3H)-one (3BDO) induced human embryonic stem cells (hESCs) differentiation. (A) Morphological images of hESCs treated with different concentrations of 3BDO obtained by using a phase-contrast microscope (200 $\times$). hESCs were treated or not with 30 or 60 μ M 3BDO for 72 h. (B) Quantified real-time polymerase chain reaction (PCR) analysis of mRNA levels of octamer-binding transcription factor 4 (Oct4), Nanog, and sex determining region Y (SRY)-box 2 (Sox2) in hESCs treated with 30 or 60 μ M 3BDO for 72 h. *GAPDH* was an internal control. (* $P < 0.05$, ** $P < 0.01$ compared with control, $n = 3$) (C) Western blot analysis of the protein levels of Oct4, Nanog, or Sox2 in hESCs with 30 or 60 μ M 3BDO for 72 h (normalized to ACTB level). * $P < 0.05$, ** $P < 0.01$ compared with control, $n = 3$.

FLJ11812 (Fig. 4B). Therefore, 3BDO could specifically decrease *FLJ11812* RNA level in hESCs.

To confirm the role of *FLJ11812* in maintaining stemness of hESCs, we examined the changes in *FLJ11812* RNA level during hESCs differentiation. *FLJ11812* RNA level was reduced in embryoid bodies (EBs) from hESCs as compared with undifferentiated hESCs (Fig. 4C). The reduction of *FLJ11812* RNA level in EBs indicated that *FLJ11812* expression was positively related with the pluripotent state. Thus, according to these results, the 3BDO-induced differentiation of hESCs might be due to the selectively decreased *FLJ11812* RNA level.

FLJ11812 functioned as an endogenous sponge of miR-4459 in hESCs, which directly bind with the 3'UTR of CDC20B

FLJ11812 acts as an endogenous "sponge" of *miR-4459* and downregulates *miR-4459* expression levels in HUVECs

FIG. 2. 3BDO downregulated core transcript factors Oct4, Sox2, and Nanog in hESCs. Immunofluorescent assay of Oct4, Nanog, or Sox2 protein level in hESCs with or without 3BDO (30 or 60 μ M) for 72 h (scale bar = 40 μ m).

FIG. 3. hESCs lost the ESC-specific cell cycle and differentiated into three layers after 3BDO treatment. **(A)** Flow cytometry of cell cycle distribution of hESCs with or without 3BDO (30 or 60 μ M) for 72 h. Image is representative of three separate experiments. **(B)** Real-time PCR analysis of mRNA levels of markers for three germ layers and self-renewal in hESCs treated or not with 3BDO (30 or 60 μ M) for 72 h. Results are from three independent experiments. *GAPDH* was an internal control. The mRNA level in untreated cells was set to 1. * P < 0.05, ** P < 0.01 compared with control, n = 3.

FIG. 4. 3BDO treatment selectively decreased *FLJ11812* RNA level in hESCs. **(A)** Quantified real-time PCR analysis of RNA levels of *FLJ11812* and mRNA levels of transforming growth factor, beta 2 (*TGFB2*) in hESCs with or without 3BDO (30 or 60 μ M) for 72 h. *GAPDH* was an internal control. ** $P < 0.01$, $n = 3$; N.S., not significant. **(B)** Fluorescent in situ hybridization of RNA levels of *FLJ11812* in hESCs with 30 or 60 μ M 3BDO for 72 h. **(C)** Real-time PCR analysis of *FLJ11812* RNA levels in undifferentiated hESCs and embryoid bodies (EBs). *GAPDH* was an internal control. ** $P < 0.01$ compared with hESCs, $n = 3$ (related to Fig. 3).

[6], so we evaluated the level of *miR-4459* in hESCs after 3BDO inhibition of *FLJ11812* RNA level. The mRNA level of *miR-4459* was increased with 3BDO treatment (Fig. 5A). Therefore, *FLJ11812* also functioned as an endogenous sponge of *miR-4459* in hESCs.

We had identified *ATG13* as a target of *miR-4459* in our previous work [6]. An miRNA may have many different mRNA targets [15]. To find out other target genes of *miR-4459*, we analyzed the 3'UTR of each predicted target mRNA sequence except *ATG13* by using RNAhybrid software, and found that the 3'UTR of *CDC20B* showed a highly conserved *miR-4459*-predicted binding sequence (Fig. 5B). The approximate 200 bp fragment of 3'UTR, including the best predicted binding sites of *miR-4459* from *CDC20B*, was cloned into the pGL3 vector. Luciferase assay showed that *miR-4459* mimics greatly reduced the luciferase activities of the reporter vectors containing the 3'UTR of *CDC20B* (Fig. 5C). Together, these results indicated that *FLJ11812* was able to downregulate *miR-4459* expression in hESCs; in addition, *CDC20B* was another downstream target of *miR-4459*.

miR-4459 regulated by FLJ11812 participates in the maintenance of hESCs stemness through decreasing the protein level of CDC20B and ATG13

To investigate the regulatory role of *FLJ11812* in the expression of *CDC20B* and *ATG13*, targets of *miR-4459*, after downregulation of *FLJ11812* by 3BDO in hESCs, we measured the protein levels of *CDC20B* and *ATG13* with or without 3BDO treatment. The result showed that the protein levels of *CDC20B* and *ATG13* were decreased in hESCs treated with 3BDO (Fig. 6A). Due to the distinctiveness of hESCs, we further overexpressed *FLJ11812* in HEK293T with or without 3BDO treatment and founded that *FLJ11812* could eliminate the effect of 3BDO treatment (Supplementary Fig. S1). These data suggested that *FLJ11812* could regulate the expression of *CDC20B* and *ATG13* by binding with *miR-4459*, which targets their 3'UTR.

FIG. 5. *miR-4459* was upregulated after 3BDO treatment and directly bound with the 3' untranslated regions (UTR) of *CDC20B*. **(A)** Real-time PCR analysis of *miR-4459* level in hESCs with or without 3BDO for 72 h, * $P < 0.05$ compared with control, $n = 3$. **(B)** Potential sites in *CDC20B* targeted by *miR-4459*. **(C)** Luc-*CDC20B* 3'UTR plasmid was transfected into HEK293 cells with negative control miRNA or *miR-4459* mimics (10 or 50 nM) for 24 h, and luciferase activity was measured, * $P < 0.05$, ** $P < 0.01$ compared with control, $n = 3$.

FIG. 6. *miR-4459* regulated the maintenance of hESCs stemness via its targets—*CDC20B* and *ATG13*. (A) Western blot analysis of protein levels of *CDC20B* and *ATG13* in hESCs with or without 3BDO (30 or 60 μ M) for 72 h (normalized to ACTB level). * P < 0.05, ** P < 0.01 compared with control, n = 3. (B) Western blot analysis of protein levels of Oct4, *CDC20B*, and *ATG13* in *miR-4459*-transduced hESCs for 24 h (normalized to ACTB level) * P < 0.05, ** P < 0.01, n = 3.

To verify whether *miR-4459* participates in the maintenance of stemness of hESCs, we detected the protein levels of its targets and Oct4 after transduction of *miR-4459* mimics. Western blot showed that *miR-4459* mimics significantly inhibited protein levels of *CDC20B* and *ATG13* as well as Oct4 (Fig. 6B). Meanwhile, we predicted the potential binding site of *miR-4459* in the whole sequence of Oct4 and did not find any binding site. Oct4 is critically involved in the self-renewal of undifferentiated ESCs and frequently used as a marker for undifferentiated cells [16]. Therefore, these results indicated that *miR-4459* indeed directly regulate the expression of *CDC20B* and *ATG13*, and indirectly regulate pluripotency in hESCs. Taken together, *miR-4459*, which is downregulated by *FLJ11812*, plays an important role in the maintenance of hESCs stemness through decreasing the protein level of *CDC20B* and *ATG13*.

Meanwhile, we previously found that 3BDO could inhibit autophagy via a *FLJ11812*–*miR-4459*–*ATG13* signal pathway in HUVECs. To validate this regulatory mechanism in hESCs, we detected autophagy by western blot and immunofluorescence in hESCs after treatment with 3BDO for 72 h. The protein level of LC3B-II and number of LC3B puncta were decreased with 3BDO treatment (Supplementary Fig. S2). Furthermore, we also found that 3BDO-treated hESCs lost the ESC-specific cell cycle. Therefore, 3BDO could inhibit cell cycle and autophagy in hESCs (Fig. 7).

Discussion

The uaRNAs have important roles in various biological processes, especially development. We recently identified a chemical molecule, 3BDO, that could efficiently induce hESCs differentiation into three layers, and selectively and significantly downregulate a uaRNA, *FLJ11812*. By acting as a ceRNA, *FLJ11812* had positive roles in the pluripotent state and maintenance of hESCs stemness. *FLJ11812* could bind with *miR-4459* to regulate the expression of its targets, *CDC20B* and *ATG13*, and thus control stemness via different biological effects.

In our previous study, 3BDO was identified as a new activator of MTOR in HUVECs [6]. MTOR also played an intricate role in the maintenance of hESCs stemness. Inhibition of MTOR impairs pluripotency by rapamycin treatment, prevents cell proliferation, and enhances mesoderm and en-

doderm activities in hESCs [17]. However, MTOR-mediated activation of p70 S6K induces differentiation of hESCs [18]. Therefore, our findings provided new clues and a powerful tool for investigating the dual role of MTOR in the maintenance of hESCs stemness.

FIG. 7. A conceptual schematic of *FLJ11812*-related regulatory mechanism in hESCs stemness. *FLJ11812* functions as a competing endogenous RNA (ceRNA) to bind with *miR-4459*, which regulates the expression of its targets (*CDC20B* and *ATG13*), thus controlling hESCs stemness by regulating the cell cycle and autophagy.

ncRNAs could function as ceRNAs in regulating the concentration and biological functions of miRNAs in stem cells [8,19]. In previous research, *linc-MD1* was found to be a ceRNA that controls muscle differentiation by binding *miR-133* and *miR-135* and regulates mRNA targets of miRNAs, including *MAML1* and *MEF2C* [19]. A large intergenic ncRNA, *lincRNA-RoR*, was identified as a ceRNA that regulates the expression of core transcript factors (Oct4, Sox2, and Nanog) and differentiation-related miRNAs, including *miR-145-5p*, *miR-181a-5p*, and *miR-99b-3p*, in hESCs [8]. *linc-RoR* shared these miRNAs with the core transcript factors and “directly” regulated self-renewal and pluripotency of hESCs through miRNAs targeting core transcript factors. This work showed that indirect regulation of a ceRNA, *FLJ11812*, regulated *miR-4459* and its targets in the stemness of hESCs.

Here, we identified that the uaRNA *FLJ11812* has ceRNA activity for *miR-4459* and regulated its targets (*CDC20B* and *ATG13*), thereby affecting the stemness of hESCs. We speculated that the two targets of *miR-4459* coregulated the hESCs stemness and differentiation: *CDC20B* and *ATG13*. *CDC20B* protein belongs to the WD repeat *CDC20/Fizzy* family and may participate in cell cycle progression. *ATG13* is required for phagosome formation and autophagy induction [20]. Some reports have shown that autophagy plays a crucial role in maintaining self-renewal and pluripotency capacity and differentiation of ESCs [21,22]. In addition to *CDC20B* and *ATG13*, there may be some other targets of *miR-4459* that are detectable through RNAhybrid software analysis, such as *TPPP* and *LARP1*, that was found to be required for spermatogenesis and embryogenesis [23,24].

Taken together, *FLJ11812* regulated the stemness of hESCs through two different biological processes—cell cycle and autophagy—by controlling *miR-4459* (Fig. 7). Our findings illuminate the different mechanisms of ceRNA in stemness regulation of hESCs.

A previous report demonstrated that *FLJ11812* was expressed in the whole embryo, mainly in the head [7], and may be important for embryogenesis and neurogenesis. However, because of overlapping *TGFB2* mRNA, *FLJ11812* is difficult to study by common RNAi. In this work, by using a chemical small molecule, 3BDO, that could selectively downregulate *FLJ11812*, we showed that *FLJ11812* played a key role in maintenance of stemness of hESCs for the first time. Therefore, our findings provide new clues and a powerful tool for investigating the action mechanism of *FLJ11812* in early development.

Conclusion

We identified 3BDO as a new tool for hESCs differentiation via selectively downregulating a uaRNA level—*FLJ11812*. *FLJ11812* functions as an endogenous sponge of *miR-4459* in hESCs, further upregulated the protein level of its targets—*CDC20B* and *ATG13*, so as to maintain stemness of hESCs through two different biological processes—cell cycle and autophagy.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (nos. 31270877, 81321061, 91313303, 20972088, and 31070735), the National 973 Research Project (no. 2011CB503906), and Specialized Research

Fund for the Doctoral Program of Higher Education (no. 20120131130010).

Author Disclosure Statement

The authors indicate no potential conflicts of interest.

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Received for publication July 15, 2014

Accepted after revision November 29, 2014

Prepublished on Liebert Instant Online December 1, 2014