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MicroRNA 433 regulates nonsense-mediated mRNA decay by targeting *SMG5* mRNA

Yi Jin^{1,2†}, Fang Zhang^{1†}, Zhenfa Ma¹ and Zhuqing Ren^{1,2*}

Abstract

Background: Nonsense-mediated mRNA decay (NMD) is a RNA quality surveillance system for eukaryotes. It prevents cells from generating deleterious truncated proteins by degrading abnormal mRNAs that harbor premature termination codon (PTC). However, little is known about the molecular regulation mechanism underlying the inhibition of NMD by microRNAs.

Results: The present study demonstrated that miR-433 was involved in NMD pathway via negatively regulating *SMG5*. We provided evidence that (1) overexpression of miR-433 significantly suppressed the expression of *SMG5* ($P < 0.05$); (2) Both mRNA and protein expression levels of *TBL2* and *GADD45B*, substrates of NMD, were increased when *SMG5* was suppressed by siRNA; (3) Expression of *SMG5*, *TBL2* and *GADD45B* were significantly increased by miR-433 inhibitor ($P < 0.05$). These results together illustrated that miR-433 regulated NMD by targeting *SMG5* mRNA.

Conclusions: Our study highlights that miR-433 represses nonsense mediated mRNA decay. The miR-433 targets 3'-UTR of *SMG5* and represses the expression of *SMG5*, whereas NMD activity is decreased when *SMG5* is decreased. This discovery provides evidence for microRNA/NMD regulatory mechanism.

Keywords: miR-433, *SMG5*, NMD

Background

Nonsense-mediated mRNA decay (NMD) recognizes and degrades mRNAs that contain premature termination codon (PTC). This surveillance mechanism could prevent from generating deleterious truncated proteins [1, 2]. UPF1 is an essential factor for NMD, which can recognize abnormal translation termination, and activate NMD [3]. Previous studies have indicated that suppressor with morphogenetic effect on genitalia 5 (*SMG5*) was involved in de-phosphorylation of UPF1 [4, 5]. *SMG5* and 7 shared a conserved 14-3-3 like domain that could recognize the phosphorylated UPF1 [6]. When UPF1 was de-phosphorylated, *SMG5* and 7 were recruited to

the P-bodies [7]. Phosphorylated serine 1096 of UPF1 could bind the heterodimer formed by *SMG5*-7 [8]. In addition this heterodimer could increase affinity combining to UPF1 [9]. *SMG5*-7 and protein phosphatase 2A (PP2A) plays an important role in the recycling for UPF1 [10, 11]. Furthermore, *SMG5*-7 interacts with deadenylases CCR4-NOT complex [12], and *SMG5* was associated to the decapping enhancer Prorich nuclear receptor co-activator 2 (PNRC2) recruiting the general decapping complex [13]. As the consequence, it is very important to investigate the expression and regulation of *SMG5* for further understanding of NMD. One recent study indicated that DEAD box protein Ddx5 regulated *SMG5* expression [14]. However, few studies have reported that microRNAs could regulate *SMG5* expression. Here we demonstrated that a microRNA, miR-433, regulated *SMG5* expression. Shih et al indicated that miR-433 was associated to poorer survival in ovarian cancer [15]. MiR-433 expression was altered in gastric cancer [16], lung

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dysplasia and myeloproliferative neoplasms [17, 18]. MiR-433 could also regulate the expression of *SFRP2*, *GRB2*, *CREB1*, and *HDAC6*, all of which were closely associated to cancer [19–23].

Two recent studies reported that miR-128, miR-125a, and miR-125b regulated NMD through silencing *UPF1* and *SMG1* [24, 25]. In present study, we demonstrated that miR-433 repressed the expression of *SMG5*, thereby suppressed NMD activity. Additionally, there may be a miR-433/NMD regulatory circuit in eukaryotic cells.

Results

MiRNA-433 targets 3'-UTR of *SMG5*

We selected the possible miRNAs targeting *SMG5* through miRNA algorithm TargetScan (<http://www.targetscan.org/>), miRanda (<http://www.microrna.org/microrna/home.do>), RNAhybrid (<http://www.bibiserv.techfak.uni-bielefeld.de/rnahybrid/>), and FINDTAR (<http://www.bio.sz.tsinghua.edu.cn/>). The prediction of all the softwares indicates that miR-433 targets *SMG5* (Fig. 1a). The 3'-UTR of *SMG5* holds a sequence motif (AUCAUGA) that is identical to the seed sequence UAGUACU (UAGUACU) of *miR-433* (Fig. 1a). We performed the dual-luciferase assay to investigate miR-433 targeting *SMG5*. The wild report gene vector pmirGLO-*SMG5*-WT and miR-433 mimic were co-transfected into HEK-293T cells. Then we detected the normalized luciferase values, which showed 50 % reduction compared with controls ($P < 0.01$, Fig. 1b). However, this regulation was abrogated when a four-nucleotide mutation (AUGA-UACU) was introduced in the miR-433 seed sequence in the 3'-UTR of *SMG5* (Fig. 1b). Additionally, the expression level of luciferase in *SMG5* mutant was increased compared to *SMG5* WT in the presence of NC (Fig. 1d). Furthermore, the dual-luciferase vectors and miR-433 inhibitor were co-transfected into Hela cells. The result showed *SMG5* expression was increased (Fig. 1c). This regulation was also abrogated when the pmirGLO-*SMG5*-MUT was transfected (Fig. 1c). In general, the results together showed that miR-433 targets 3'-UTR of *SMG5* directly.

The suppression of *SMG5* repressed NMD activity

The previous studies have indicated that *SMG5* was an important NMD factor, so we are going to suppress *SMG5* expression by RNAi to confirm this function. The siRNA was transfected into C2C12 cells for 24–48 h. The results showed that *SMG5* was decreased in both mRNA and protein expression level (Fig. 2a, b). According to the NMD mechanism, if NMD activity reduced, the expression of NMD substrates would be reduced. The NMD activity thereby was indicated by the expression level of NMD substrates, *TBL2* and *GADD45B*. We detected the

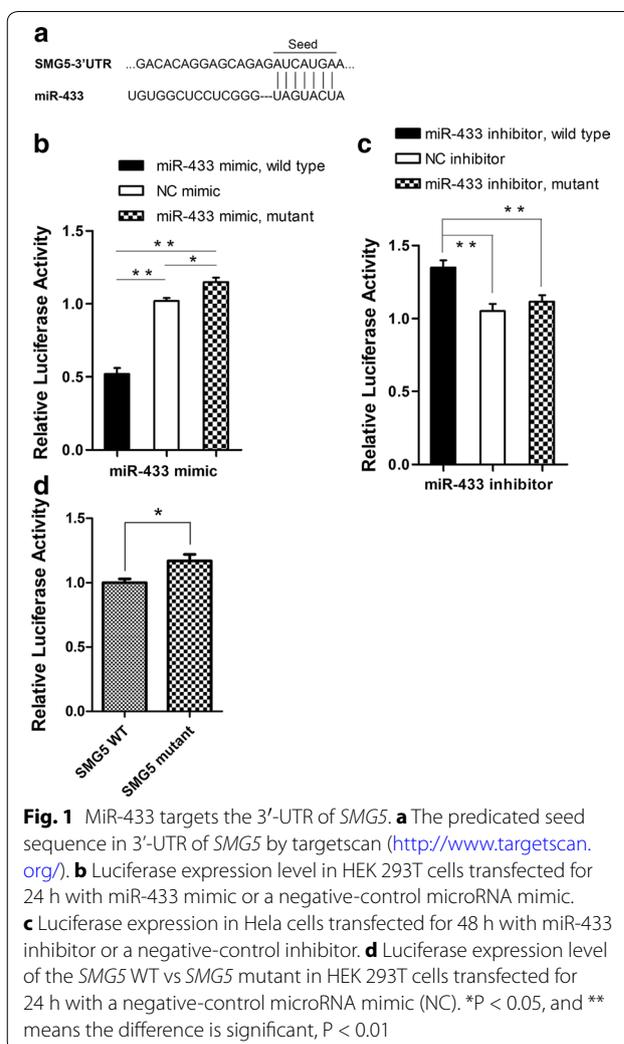


Fig. 1 MiR-433 targets the 3'-UTR of *SMG5*. **a** The predicted seed sequence in 3'-UTR of *SMG5* by targetscan (<http://www.targetscan.org/>). **b** Luciferase expression level in HEK 293T cells transfected for 24 h with miR-433 mimic or a negative-control microRNA mimic. **c** Luciferase expression in Hela cells transfected for 48 h with miR-433 inhibitor or a negative-control inhibitor. **d** Luciferase expression level of the *SMG5* WT vs *SMG5* mutant in HEK 293T cells transfected for 24 h with a negative-control microRNA mimic (NC). * $P < 0.05$, and ** means the difference is significant, $P < 0.01$

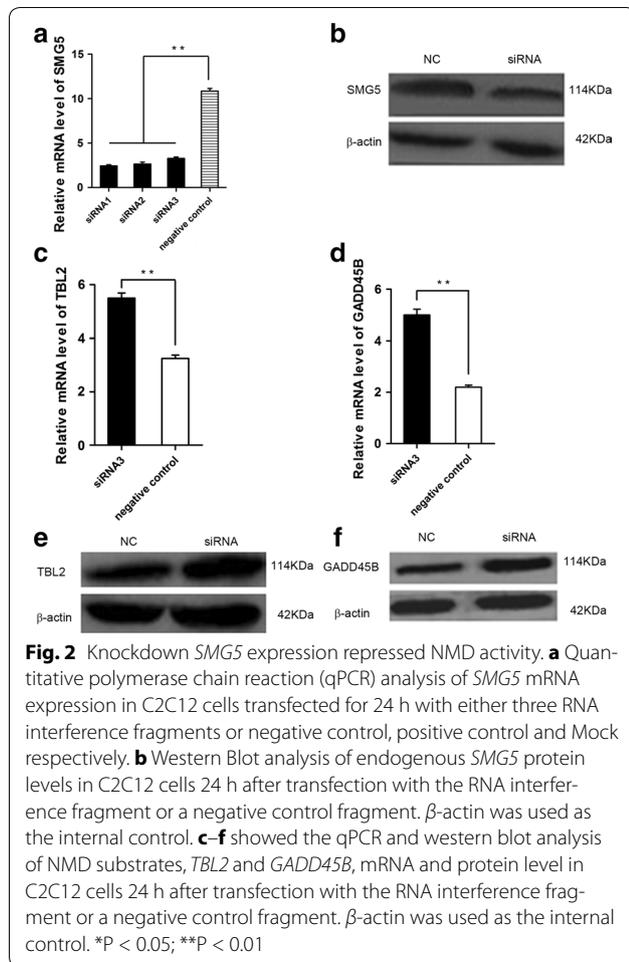
expression level of *TBL2* and *GADD45* and found that both of them were increased ($P < 0.05$, Fig. 2c–f). Therefore the RNAi experiment indicated that suppression of *SMG5* repressed NMD activity.

MiR-433 repressed *SMG5* expression

When we transfected miR-433 mimic into BHK cells, the expression level of *SMG5* was decreased, ($P < 0.01$, Fig. 3). Furthermore, the expression level of *SMG5* was increased significantly when the miR-433 inhibitor was transfected into the BHK cells ($P < 0.01$, Fig. 3). As the consequence, the *SMG5* expression was down-regulated by miR-433.

MiR-433 repressed NMD activity

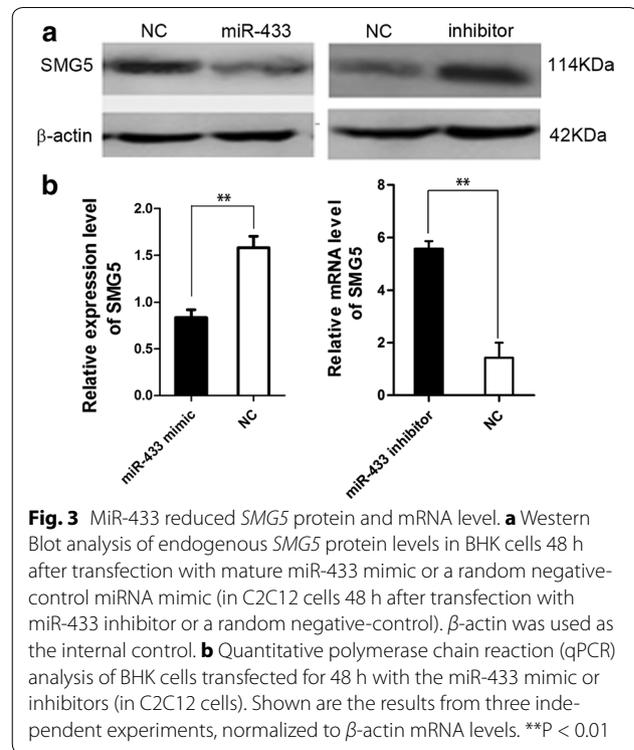
Since we have demonstrated that miR-433 repressed *SMG5* expression and suppression of *SMG5* repressed NMD activity, miR-433 would repress NMD activity. In



this section, the results showed that the NMD substrates *TBL2* and *GADD45B* were increased in BHK cells when transfected with miR-433 mimic ($P < 0.05$, Fig. 4). In addition, we chose another cell line (C2C12) to transfect miR-433 inhibitor. The expression level of *TBL2* and *GADD45B* were also decreased by miR-433 inhibitor ($P < 0.05$, Fig. 4).

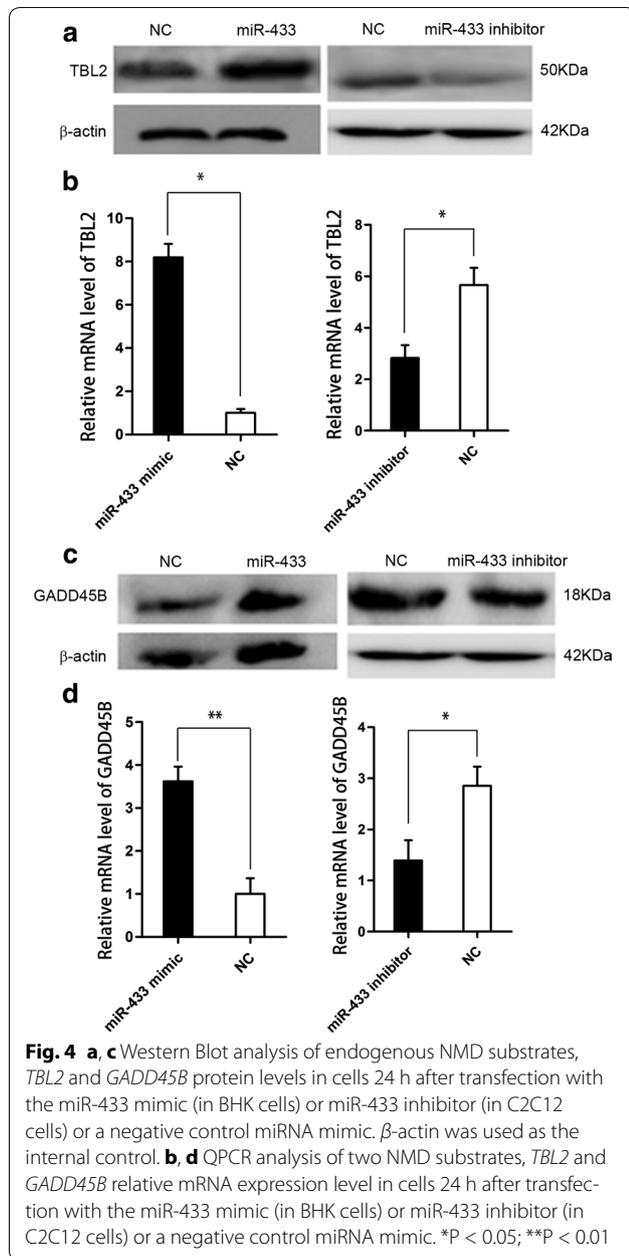
Discussion

Nonsense-mediated mRNA decay, a surveillance system, which recognizes mRNAs with translation termination codons positioned in abnormal contexts and degrades aberrant mRNAs, scrutinizes mRNA quality in all mammalian cells [26]. In this intricate process, a set of NMD factors are influential to the recognition and degradation of aberrant mRNAs. *UPF1*, the master regulator of NMD, is considered to determine the NMD process [27–29]. The ATPase and helicase activities as well as the phosphorylation of N- and C- terminal domains bestow on *UPF1* ability that recognizes premature termination codon containing mRNAs selectively [30–33]. The phosphorylation and de-phosphorylation of *UPF1* were



very important for NMD. *SMG5*, an important NMD factor, played an important role in *UPF1* de-phosphorylation process. As our results showed, NMD activity was repressed when the expression of *SMG5* was suppressed. So the regulation for *SMG5* could affect NMD activity. We selected a microRNA, miR-433, which targets 3'-UTR of *SMG5* by prediction. The regulation of miR-433 targeted *SMG5* was detected by dual-luciferase report assay. The result indicated that *SMG5* expression was reduced by 50 % when we transfected with miR-433 mimic. It has demonstrated that the NMD activity was repressed when *SMG5* was suppressed (Fig. 3). Hence, miRNA-433 repressed NMD activity by suppressing *SMG5* expression.

As a conserved mRNA surveillance system in eukaryotic cells, NMD is crucial for many physiological processes, and these crucial function of NMD have been published by many researches. Numbers of NMD factors were important for embryo development. When *UPF1*, *UPF2*, *SMG1* or *SMG6* was knockout or knock-down technologically, all mice died during an early embryonic stage [34–37]. A study implied that NMD has the potency that affects cell type diversification [34]. NMD activity was identified to be reduced in neuronal stem cells differentiation process. And this regulation of NMD activity is essential for nervous system development [38]. Additionally, NMD also regulates neural



development related genes [39, 40]. Here, our experiment indicated that miR-433 repressed *SMG5* expression, and therefore suppressed NMD activity. This result indicated a circuit regulation of miR-433 and NMD. Hence, miR-433 could participate in the process regulated by NMD. For the circuit regulation, previous study identified miR-128 repressed NMD by *UPF1* and *MLN51* [24]. MiR-125 and 128 were involved in many nervous system disorder diseases such as autism [41], prion-induced neuron degeneration [42], Huntington's disease [43], Parkinson's disease [44], as well as Alzheimer's disease [45]. Some studies indicated that NMD

played an important role in brain development and embryonic development [46, 47], and three essential embryonic brain vesicles formation disrupted by knock-out of *SMG1* [48]. Therefore, we assumed that there may be a miR-433/NMD regulatory circuit in early embryo development, especially in early nervous and brain development. This study would provide evidence to make clear the microRNA/NMD regulatory mechanism and give advice to neurological disease and related cancers therapies.

Conclusion

It is well known that nonsense mediated mRNA decay is an important mRNA quality surveillance system. However, the microRNA/NMD regulatory mechanism is not fully clear. Our study claim that NMD activity is repressed by a microRNA, miR-433. The miR-433 targets 3'UTR of *SMG5* and decreases the expression of *SMG5*. NMD activity is suppressed when expression of *SMG5* is reduced. This discovery provides evidence for microRNA/NMD regulatory mechanism and therapeutic advice for NMD-related diseases.

Methods

Cell culture

The cell lines, HEK-293T, HeLa, BHK-21, and C2C12, were purchased from China for type culture collection (CCTCC). Cells expect for HeLa were maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Logan, Utah, USA) supplemented with 10 % fetal bovine serum (FBS, Hyclone, Logan, Utah, USA) at 37 °C in a humidified atmosphere of 5 % CO₂. HeLa cells were maintained in DMEM (Hyclone, Logan, Utah, USA) containing 20 % FBS (Hyclone, Logan, Utah, USA) at 37 °C in a humidified atmosphere of 5 % CO₂. Medium was changed twice weekly until the fibroblasts migrated out to cover the dishes. Passage 6–9 cells were used for all experiments.

Isolation of 3'-UTR sequences of *SMG5*

C2C12 genome DNA was used as template to amplify 3'-UTR of *SMG5*. To isolate the 464-bp 3'-UTR of 2.6-kb genome, the following primer pairs were used: *SMG5*-3'UTR-F and *SMG5*-3'UTR-R [5'-cgagctcCTG TACTGGATAAAGGGTGCC-3' and 5'-ccgctcgcagCA ACTGTTCCCTGGTTTTCC, with uppercase bases corresponding to the mouse 2.6-kb *SMG5* genome and lowercase bases indicating 5' extensions with restriction enzyme sites (underline) for *SacI* and *XhoI*, respectively]. The protocol was as follows: pre-denaturation at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 64 °C for 30 s, elongation at 72 °C for 30 s per 1 kb, thirty-five cycles, elongation at 72 °C for 2 min, and end.

PCR products were investigated by 1–2 % agarose gel electrophoresis. The restriction enzyme, *SacI* and *XhoI* were purchased from Fermentas (Thermo Fisher Scientific, Rockford, IL, USA).

Wild type report gene constructs

The 2.2. PCR product was purified and cloned into the *SacI* and *XhoI* sites of pmirGLO dual-luciferase miRNA target expression vector (Promega, Madison, WI, USA). The enzyme digestion system contained 10× buffer 4 μL, *SacI* 4 μL, *XhoI* 4 μL, pmirGLO vector 12 μL, and the ddH₂O up to 40 μL. The enzyme digestion system was incubated at 37 °C for 2 h. The T₄ DNA ligase (TaKaRa) interaction system contained enzyme digestion fragment 3 μL, digested pmirGLO plasmid 2 μL, Solution I 5 μL. The system was incubated at 16 °C for 2 h. The accuracy of inserted 3'-UTR of *SMG5* were confirmed by sequencing. Then the plasmid was isolated and purified using E.Z.N.A.[®] endo-free plasmid mini kit II (OMEGA, Bio-Tek, Norcross, GA, USA). The constructed wild type report gene recombinants named pmirGLO-SMG5-WT was stored at −20 °C.

Introducing four-nucleotide mutagenesis into *SMG5* 3'-UTR

Two primer pairs, SMG5-3'UTR-F (5'-cgagctcCTGTA CTGGATAAGGGGTGCC-3') and SMG5-3'UTR-R (5'-ccgctcgagCAACTGTTCCCTGTTTTCCC), SMG5-3'UTR-MF (5'-AGCAGAGATCTACTATACTC AG-3') and SMG5-3'UTR-MR (5'-GCCCTGAGT ATAGTAGATCTCTG-3'), were designed (uppercase bases corresponding to the mouse 2.6-kb *SMG5* genome and lowercase bases indicating 5' extensions with restriction enzyme sites [underline] for *SacI* and *XhoI*, respectively. And the bold bases mean four-nucleotide mutation sites of *SMG5* 3'-UTR). The former was used to construct pmirGLO-SMG5-WT, whereas the latter to amplify the mutation sites and its upstream sequence (production I), and the mutation sites and its downstream sequence (production II) respectively, the pmirGLO-SMG5-WT plasmid was the template. Then using fusion PCR combined these two products. The PCR system contained 2× taq PCR mix 11 μL, recovered production I 2 μL, recovered production II 2 μL, ddH₂O 10 μL. PCR conditions: an initial denaturation at 95 °C for 4 min, followed by 30 cycles of 95 °C for 30 s, 40 °C for 30 s, 45 °C for 5 s, 50 °C for 5 s, 55 °C for 5 s, 60 °C for 5 s and 72 °C for 30 s, with a final extension of 5 min at 72 °C. PCR products were visible after electrophoresis of 25-μL reaction using an agarose gel (1.5 %). Then the fusion PCR production was recovered and used as a template.

Mutant report gene constructs

The 464 bp mutant *SMG5* 3'-UTR was amplified, purified, and cloned into the *SacI* and *XhoI* sites of pmirGLO dual-luciferase miRNA target expression vector (Promega, Madison, WI). The enzyme digestion system contained 10× buffer 4 μL, *SacI* 4 μL, *XhoI* 4 μL, pmirGLO vector 12 μL, and ddH₂O up to 40 μL. The enzyme digestion system was incubated at 37 °C for 2 h. The T₄ DNA ligase (TaKaRa) interaction system contained enzyme digestion fragment 3 μL, digested pmirGLO plasmid 2 μL, Solution I 5 μL. This system was incubated at 16 °C for 2 h. The accuracy of inserted *SMG5* 3'-UTR was confirmed by sequencing. Then the plasmid was isolated and purified using E.Z.N.A.[®] endo-free plasmid mini kit II (OMEGA, Bio-Tek, Norcross, GA, USA). The mutant report gene vector, named pmirGLO-SMG5-MUT conserved at −20 °C.

Cell transfection and dual-luciferase assay

HEK-293T cells were seeded onto 24-well plates at 30–40 % confluence. The next day, medium was changed to Opti-MEM I Reduced Serum Medium (Hyclone, Logan, Utah, USA), and cells were transfected with pmirGLO-SMG5-WT constructs (200 ng per well). After 4–6 h, medium was replaced by MEM containing 10 % FBS (Hyclone, Logan, Utah, USA). Transfected cells were washed twice with cold PBS (Hyclone, Logan, Utah, USA), lysed using 1× passive lysis buffer (Promega) for 30 min, and assayed for firefly and Renilla luciferase activities by the dual-luciferase assay (Promega) in a PerkinELmer 2030 Microplate Reader (Lenovo, China) according to the manufacturer's instructions. At least three independent experiments were performed for each assay, each time with a minimum of n = 4. For co-transfection, cells were grown on 24-well plates. For each well, a transfection mixture (200 μL) consisting of 0.2-μg reporter construct, 1.25 μL miR-433 mimic or negative control, and 2 μL Lipofectamine 2000 Reagent (Invitrogen) was prepared. Opti-MEM I Reduced Serum Medium was used as diluent. Hella cells were treated as same as the HEK-293T, but the medium was MEM containing 20 % FBS.

RNA isolation and mRNA/miRNA quantitation

The RNA isolation reagent was TRizol[®] Reagent (Invitrogen, Carlsbad, CA, USA). RNA was quantified spectroscopically (ND-1000 Spectrophotometer; NanoDrop, Wilmington, DE, USA), and integrity was assessed by agarose gel electrophoresis. According to the manufacturer's protocol, RNA was reverse-transcribed using RNA PCR kit (Takara Bio Inc.). The gene expression was

investigated by RT-PCR. Primers for RT-PCR were as follows: *SMG5*-qF (5'-TACCTCATCCCTGACACCCA-3') and *SMG5*-qR (5'-GCCCTGGCTGTTCTTTCT-3'), miR-433-qF (CTGGTAGGATCATGATGGGAT) and miR-433-qR (TCAACTGGTGTCTGAGT), *U6*-qF (CTGGTAGGGTCTCGCTTCGGCAG) and *U6*-qR (CAACTGGTGTCTGAGTTCGGC), *TBL2*-qF (5'-AA GTATCTGGCCACCTGTGC-3') and *TBL2*-qR (5'-GG CCAGCCAAACAATGAAGG-3'), *GADD45B*-qF (5'-AA GGCGGCCAAACTGATGAAT-3') and *GADD45B*-qR (5'-ATTGTCGCAGCAGAACGACT-3'), β -*actin*-F (5'-G CCTCACTGTCCACCTTCCA-3') and β -*actin*-R (5'-A GCCATGCCAATGTTGTCTCTT-3'). All the primer pairs for each gene were used for gene-specific amplification. The expression of β -*actin* was the internal control. For quantification, the comparative threshold cycle method was used to assess relative changes in mRNA levels [49].

MicroRNA overexpression assay

MiR-433 mimic and inhibitor were purchased from GenePharma (shanghai, China). MiR-433 mimic could enhance endogenous miR-433 function, and miR-433 inhibitor could repress the endogenous miR-433 function. HK-21 cells and C2C12 cells were seeded onto 6-well plates in DMEM supplemented with 10 % FBS at 37 °C in a humidified atmosphere of 5 % CO₂. The next day, when the cells confluence get 70–80 %, the medium was changed to Opti-MEM I Reduced Serum Medium (Hyclone, Logan, Utah, USA), and cells were transfected with 10 μ L miRNA mimic (0.02 nmol/ μ L) using Lipofectamine 2000 Reagent (Invitrogen). After 4–6 h, medium was replaced by MEM containing 10 % FBS (Hyclone, Logan, Utah, USA). Transfected cells were washed twice with cold PBS (Hyclone, Logan, Utah, USA). At least three independent experiments were performed for each assay, each time with a minimum of $n = 4$. Opti-MEM I Reduced Serum Medium was used as diluent. The mimic was added fluorescent tag so that it could be detected under fluorescence microscope to calculate the transfection efficiency.

Small interfering RNA design and cell transfection

The *musSMG5* mRNA sequence and internal control (β -*actin*) mRNA sequences were obtained from NCBI database (<http://www.ncbi.nlm.nih.gov/>). The siRNAs were purchased from Genpharma (Inc. Shanghai, China). The sequences of siRNAs were as follows: siRNA1 5'-GCCGCUUCAUCAUCAUTT-3' for sense and 5'-AUGAUGAUGAUGAAGCGGCTT-3' for antisense; siRNA2 5'-GGAGUGUGAAAGUGGAUUA TT-3' for sense and 5'-AUAUCCACUUUCACACU CCTT-3' for antisense; siRNA3 5'-GCAGGCAGCAA

GUAUUACATT-3' for sense and 5'-UGUAAUACUU GCUGCCUGCTT-3' for antisense; NC (negative control) 5'-UUCUCCGAACGUGUCACGUTT-3' for sense and 5'-ACGUGACACGUUCGGAGAATT-3' for antisense. C2C12 cells were incubated in 5 % CO₂ at 37 °C in DMEM (Hyclone, Logan, Utah, USA) supplemented with 10 % FBS (Hyclone, Logan, Utah, USA). One day before transfection, 10⁵ cells were plated in 2 mL DMEM containing 10 % FBS per well of a 6-well plate. The next day, when the cells confluence get 50 %, the medium was changed to Opti-MEM I Reduced Serum Medium (Hyclone, Logan, Utah, USA), and cells were transfected with 10 μ L siRNA using 10 μ L Lipofectamine 2000 Reagent (Invitrogen). After 4–6 h, medium was replaced by MEM containing 10 % FBS (Hyclone, Logan, Utah, USA). The cells were incubated for 24–48 h. Transfected cells were washed twice with cold PBS (Hyclone, Logan, Utah, USA). At least three independent experiments were performed for each assay, each time with a minimum of $n = 4$. Opti-MEM I Reduced Serum Medium was used as diluent.

Western blotting

The whole-cell protein of BHK-21 and C2C12 were collected using RIPA and PMSF, and conserved at -20 °C. The concentration of protein was detected using the bicinchoninic acid (BCA) method (Beyotime Biotechnology, Jiangsu, China). Subsequently, samples were separated by SDS-PAGE (10 %) and electroblotted onto polyvinylidene fluoride (PVDF) membrane (Millipore, USA). Membrane was blocked with 1 \times TBST supplemented with 5 % skimmed milk powder (servicebio, Wuhan, China). Membranes were incubated with the primary antibody, anti-SMG5 (P-14) (goat, #SC50980, Santa Cruz Biotechnology, Inc.). For normalization of the results, membranes were reblotted for β -*actin* (anti- β -*actin*, servicebio, Wuhan). Proteins were detected with secondary antibody (HRP-rabbit, anti-goat, 1:1000; booster, Wuhan, China). For the *TBL2* and *GADD45B* western blotting analysis, the primary antibodies were anti-*TBL2* (L-15) (goat, #SC-104692, Santa Cruz Biotechnology, Inc.), and anti-*GADD45B* (rabbit, #ab128920, abcam, Inc.). Bands on the X-ray films were quantified with WCIF ImageJ software for the densitometry analysis.

Statistical analysis

All data were displayed as the mean \pm SD of three independent experiments, in which each assay was performed in triplicate. Statistically significant differences between two groups were determined by Student's *t* test. $P < 0.05$ was considered statistically significant.

Abbreviations

miR-433: microRNA-433; NMD: nonsense mediated mRNA decay; SMG5: suppressor with morphological effect on genitalia 5; PTC: premature termination codon; TBL2: transducin beta-like protein 2; GADD45B: growth arrest and DNA damage-inducible protein GADD45 beta; UTR: untranslated regions; UPF1: up-framshift 1; SMG7: suppressor with morphological effect on genitalia 7; PP2A: protein phosphatase 2A; PNRC2: prorich nuclear receptor co-activator 2; SFRP2: secreted frizzled-related protein 2; GRB2: growth factor receptor-bound protein 2; CREB1: CAMP-response element binding protein; HDAC6: histone deacetylase 6.

Authors' contributions

YJ sorted and analyzed data, wrote the article. FZ designed this experiment, and performed the experiment. ZM purchased the reagents and materials for the experiment, and performed some part of the experiment. ZR designed this work and participated in the revision of this article. All authors read and approved the final manuscript.

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None.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The original data of the real-time PCR experiments, dual-luciferase assay and data images for western blot analysis will be available upon request.

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References

- Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends Biochem Sci.* 1998;23(6):198–9.
- Peltz SW, Brown AH, Jacobson A. mRNA destabilization triggered by premature translational termination depends on at least three cis-acting sequence elements and one trans-acting factor. *Genes Dev.* 1993;7(9):1737–54.
- Hilleren P, Parker R. mRNA surveillance in eukaryotes: kinetic proofreading of proper translation termination as assessed by mRNP domain organization? *RNA.* 1999;5(6):711–9.
- Chiu SY, Serin G, Ohara O, Maquat LE. Characterization of human Smg5/7a: a protein with similarities to *Caenorhabditis elegans* SMG5 and SMG7 that functions in the dephosphorylation of Upf1. *RNA.* 2003;9(1):77–87.
- Ohnishi T, Yamashita A, Kashima I, Schell T, Anders KR, Grimson A, Hachiya T, Hentze MW, Anderson P, Ohno S. Phosphorylation of hUPF1 induces formation of mRNA surveillance complexes containing hSMG-5 and hSMG-7. *Mol Cell.* 2003;12(5):1187–200.
- Fukuhara N, Ebert J, Unterholzner L, Lindner D, Izaurralde E, Conti E. SMG7 is a 14-3-3-like adaptor in the nonsense-mediated mRNA decay pathway. *Mol Cell.* 2005;17(4):537–47.
- Unterholzner L, Izaurralde E. SMG7 acts as a molecular link between mRNA surveillance and mRNA decay. *Mol Cell.* 2004;16(4):587–96.
- Okada-Katsuhata Y, Yamashita A, Kutsuzawa K, Izumi N, Hirahara F, Ohno S. N- and C-terminal Upf1 phosphorylations create binding platforms for SMG-6 and SMG-5:SMG-7 during NMD. *Nucleic Acids Res.* 2012;40(3):1251–66.
- Jonas S, Weichenrieder O, Izaurralde E. An unusual arrangement of two 14-3-3-like domains in the SMG5-SMG7 heterodimer is required for efficient nonsense-mediated mRNA decay. *Genes Dev.* 2013;27(2):211–25.
- Anders KR, Grimson A, Anderson P. SMG-5, required for *C. elegans* nonsense-mediated mRNA decay, associates with SMG-2 and protein phosphatase 2A. *EMBO J.* 2003;22(3):641–50.
- Durand S, Cougot N, Mahuteau-Betzler F, Nguyen CH, Grierson DS, Bertrand E, Tazi J, Lejeune F. Inhibition of nonsense-mediated mRNA decay (NMD) by a new chemical molecule reveals the dynamic of NMD factors in P-bodies. *J Cell Biol.* 2007;178(7):1145–60.
- Loh B, Jonas S, Izaurralde E. The SMG5-SMG7 heterodimer directly recruits the CCR4-NOT deadenylase complex to mRNAs containing nonsense codons via interaction with POP2. *Genes Dev.* 2013;27(19):2125–38.
- Cho H, Han S, Choe J, Park SG, Choi SS, Kim YK. SMG5-PNRC2 is functionally dominant compared with SMG5-SMG7 in mammalian nonsense-mediated mRNA decay. *Nucleic Acids Res.* 2013;41(2):1319–28.
- Geissler V, Altmeyer S, Stein B, Uhlmann-Schiffler H, Stahl H. The RNA helicase Ddx5/p68 binds to hUpf3 and enhances NMD of Ddx17/p72 and Smg5 mRNA. *Nucleic Acids Res.* 2013;41(16):7875–88.
- Shih KK, Qin LX, Tanner EJ, Zhou Q, Bisogna M, Dao F, Olvera N, Viale A, Barakat RR, Levine DA. A microRNA survival signature (MiSS) for advanced ovarian cancer. *Gynecol Oncol.* 2011;121(3):444–50.
- Ueda T, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, Alder H, Liu CG, Oue N, Yasui W, et al. Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. *Lancet Oncol.* 2010;11(2):136–46.
- Del Vecovo V, Meier T, Inga A, Denti MA, Borlak J. A cross-platform comparison of affymetrix and Agilent microarrays reveals discordant miRNA expression in lung tumors of c-Raf transgenic mice. *PLoS ONE.* 2013;8(11):e78870.
- Lin X, Rice KL, Buzzai M, Hexner E, Costa FF, Kilpivaara O, Mullally A, Soares MB, Ebert BL, Levine R, et al. miR-433 is aberrantly expressed in myeloid-proliferative neoplasms and suppresses hematopoietic cell growth and differentiation. *Leukemia.* 2013;27(2):344–52.
- Gotanda K, Hirota T, Matsumoto N, Ieiri I. MicroRNA-433 negatively regulates the expression of thymidylate synthase (TYMS) responsible for 5-fluorouracil sensitivity in HeLa cells. *BMC Cancer.* 2013;13:369.
- Luo H, Zhang H, Zhang Z, Zhang X, Ning B, Guo J, Nie N, Liu B, Wu X. Down-regulated miR-9 and miR-433 in human gastric carcinoma. *J Exp Clin Cancer Res.* 2009;28:82.
- Simon D, Laloo B, Barillot M, Barnettech T, Blanchard C, Rooryck C, Marche M, Burgelin I, Coupry I, Chassaing N, et al. A mutation in the 3'-UTR of the HDAC6 gene abolishing the post-transcriptional regulation mediated by hsa-miR-433 is linked to a new form of dominant X-linked chondrodysplasia. *Hum Mol Genet.* 2010;19(10):2015–27.
- Snyder CM, Rice AL, Estrella NL, Held A, Kandarian SC, Naya FJ. MEF2A regulates the Gtl2-Dio3 microRNA mega-cluster to modulate WNT signaling in skeletal muscle regeneration. *Development.* 2013;140(1):31–42.
- Yang Z, Tsuchiya H, Zhang Y, Hartnett ME, Wang L. MicroRNA-433 inhibits liver cancer cell migration by repressing the protein expression and function of cAMP response element-binding protein. *J Biol Chem.* 2013;288(40):28893–9.
- Bruno IG, Karam R, Huang LL, Bhardwaj A, Lou CH, Shum EY, Song HW, Corbett MA, Gifford WD, Gecz J, et al. Identification of a MicroRNA that activates gene expression by repressing nonsense-mediated RNA decay. *Mol Cell.* 2011;42(4):500–10.
- Wang G, Jiang B, Jia C, Chai B, Liang A. MicroRNA 125 represses nonsense-mediated mRNA decay by regulating SMG1 expression. *Biochem Biophys Res Commun.* 2013;435(1):16–20.
- Lykke-Andersen SJT. Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes. *Nat Rev Mol Cell Biol.* 2015;16:665–7.
- Kervestin S, Jacobson A. NMD: a multifaceted response to premature translational termination. *Nat Rev Mol Cell Biol.* 2012;13(11):700–12.

28. Schweingruber C, Rufener SC, Zund D, Yamashita A, Muhlemann O. Nonsense-mediated mRNA decay - mechanisms of substrate mRNA recognition and degradation in mammalian cells. *Biochim Biophys Acta*. 2013;1829(6-7):612-23.
29. Siwaszek A, Ukleja M, Dziembowski A. Proteins involved in the degradation of cytoplasmic mRNA in the major eukaryotic model systems. *RNA Biol*. 2014;11(9):1122-36.
30. Franks TM, Singh G, Lykke-Andersen J. Upf1 ATPase-dependent mRNA disassembly is required for completion of nonsense-mediated mRNA decay. *Cell*. 2010;143(6):938-50.
31. Kashima I, Yamashita A, Izumi N, Kataoka N, Morishita R, Hoshino S, Ohno M, Dreyfuss G, Ohno S. Binding of a novel SMG-1-Upf1-eRF1-eRF3 complex (SURF) to the exon junction complex triggers Upf1 phosphorylation and nonsense-mediated mRNA decay. *Genes Dev*. 2006;20(3):355-67.
32. Kurosaki T, Li W, Hoque M, Popp MW, Ermolenko DN, Tian B, Maquat LE. A post-translational regulatory switch on UPF1 controls targeted mRNA degradation. *Genes Dev*. 2014;28(17):1900-16.
33. Weng Y, Czaplinski K, Peltz SW. Genetic and biochemical characterization of mutations in the ATPase and helicase regions of the Upf1 protein. *Mol Cell Biol*. 1996;16(10):5477-90.
34. Li T, Shi Y, Wang P, Guachalla LM, Sun B, Joerss T, Chen YS, Groth M, Krueger A, Platzer M, et al. Smg6/Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay. *EMBO J*. 2015;34(12):1630-47.
35. McIlwain DR, Pan Q, Reilly PT, Elia AJ, McCracken S, Wakeham AC, Itie-Youten A, Blencowe BJ, Mak TW. Smg1 is required for embryogenesis and regulates diverse genes via alternative splicing coupled to nonsense-mediated mRNA decay. *Proc Natl Acad Sci USA*. 2010;107(27):12186-91.
36. Medghalchi SM, Frischmeyer PA, Mendell JT, Kelly AG, Lawler AM, Dietz HC. Rent1, a trans-effector of nonsense-mediated mRNA decay, is essential for mammalian embryonic viability. *Hum Mol Genet*. 2001;10(2):99-105.
37. Weischenfeldt J, Damgaard I, Bryder D, Theilgaard-Monch K, Thoren LA, Nielsen FC, Jacobsen SE, Nerlov C, Porse BT. NMD is essential for hematopoietic stem and progenitor cells and for eliminating by-products of programmed DNA rearrangements. *Genes Dev*. 2008;22(10):1381-96.
38. Lou CH, Shao A, Shum EY, Espinoza JL, Huang L, Karam R, Wilkinson MF. Posttranscriptional control of the stem cell and neurogenic programs by the nonsense-mediated RNA decay pathway. *Cell Rep*. 2014;6(4):748-64.
39. Colak D, Ji SJ, Porse BT, Jaffrey SR. Regulation of axon guidance by compartmentalized nonsense-mediated mRNA decay. *Cell*. 2013;153(6):1252-65.
40. Nguyen LS, Jolly L, Shoubridge C, Chan WK, Huang L, Laumonier F, Raynaud M, Hackett A, Field M, Rodriguez J, et al. Transcriptome profiling of UPF3B/NMD-deficient lymphoblastoid cells from patients with various forms of intellectual disability. *Mol Psychiatry*. 2012;17(11):1103-15.
41. Abu-Elneel K, Liu T, Gazzaniga FS, Nishimura Y, Wall DP, Geschwind DH, Lao K, Kosik KS. Heterogeneous dysregulation of microRNAs across the autism spectrum. *Neurogenetics*. 2008;9(3):153-61.
42. Saba R, Goodman CD, Huzarewich RL, Robertson C, Booth SA. A miRNA signature of prion induced neurodegeneration. *PLoS ONE*. 2008;3(11):e3652.
43. Lee ST, Chu K, Im WS, Yoon HJ, Im JY, Park JE, Park KH, Jung KH, Lee SK, Kim M, et al. Altered microRNA regulation in Huntington's disease models. *Exp Neurol*. 2011;227(1):172-9.
44. Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV, Murchison E, Hannon G, Abeliovich A. A MicroRNA feedback circuit in midbrain dopamine neurons. *Science*. 2007;317(5842):1220-4.
45. Lukiw WJ. Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. *NeuroReport*. 2007;18(3):297-300.
46. Wittkopp N, Huntzinger E, Weiler C, Sauliere J, Schmidt S, Sonawane M, Izaurralde E. Nonsense-mediated mRNA decay effectors are essential for zebrafish embryonic development and survival. *Mol Cell Biol*. 2009;29(13):3517-28.
47. Anastasaki C, Longman D, Capper A, Patton EE, Caceres JF. Dhx34 and Nbas function in the NMD pathway and are required for embryonic development in zebrafish. *Nucleic Acids Res*. 2011;39(9):3686-94.
48. McIlwain DR, Pan Q, Reilly PT, Elia AJ, McCracken S, Wakeham AC, Itie-Youten A, Blencowe BJ, Mak TW. Smg1 is required for embryogenesis and regulates diverse genes via alternative splicing coupled to nonsense-mediated mRNA decay. *P Natl Acad Sci USA*. 2010;107(27):12186-91.
49. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*. 2001;25(4):402-8.

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