Supplementary information

Spatial expression of the nonsense-mediated mRNA decay factors UPF3A and UPF3B among mouse tissues

Xin MA[1], Yan LI[1], Chengyan CHEN[1], Yanmin SHEN[1], Hua WANG[2], Tangliang LI[1,2]

[1]*State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, China*
[2]*Key Laboratory of Aging and Cancer Biology of Zhejiang Province, Department of Pathology and Pathophysiology, School of Basic Medical Sciences, Hangzhou Normal University, Hangzhou 311121, China*

Materials and methods

Mice

Male and female C57BL/6 mice aged 8–10 weeks were maintained under specific pathogen-free conditions at the animal facility of Shandong University (Qingdao, China). All mice had free access to water and food. All animal care and experiments were performed according to the guidelines of the ethics committee (license number: SYDWLL-2022-083).

Real-time quantitative PCR (qPCR)

Total RNAs of mouse tissues were extracted by TRIzol reagent (TaKaRa Bio, Tokyo, Japan), then were used for cDNA synthesis with HiScript® II Q Select RT SuperMix for qPCR (Vazyme Biotech Co., Ltd., Nanjing, China). RT-qPCR was performed on the CFX Real-time PCR system (Bio-Rad, Hercules, CA, USA) using SYBR Green Master qPCR Mix (Tsingke Biotechnology Co., Ltd., Beijing, China). The primers used are listed as below:

mUpf3a: F, GCGCACGATTACTTCGAGGT; R, TCAAAACGGTCTCTGAACAGC;

mUpf3b: F, AGGAGAAACGAGTGACCCTGT; R, CCTGTTGCGATCCTGCCTA;

mβ-actin: F, AGAGGGAAATCGTGCGTGAC; R, CAATAGTGATGACCTGGCCGT.

Western blotting

Western blotting analysis followed a previously published method (Li and Wang, 2011). Briefly, RIPA buffer (Radioimmunoprecipitation assay buffer) supplemented with protease/phosphatase inhibitors (APExBIO, Houston, TX, USA) was used for total protein extraction from mouse tissues. The Pierce BCA

Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used for measuring the protein concentrations. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed to separate the proteins, which were further transferred to PVDF membranes (Merck Millipore Ltd., Darmstadt, Germany). After blocking with 5% milk-Tris-buffered saline with 1× Tween 20 (TBST) for 2 h, the membranes were incubated with rabbit anti-UPF3A+3B antibody (1:1000, Abcam, Cambridge, UK) or mouse anti-GAPDH antibody (1:5000, Proteintech, Tokyo, Japan) overnight at 4 ℃, then washed three times with 1× TBST and incubated with corresponding secondary antibodies for 1 h at room temperature. Protein bands were visualized using BeyoECL Plus (Beyotime Biotechnology, Shanghai, China), and quantified using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Data were statistically analyzed by SPSS (v26.0) software, and all data are presented as means ± standard deviation (SD). *P* value of < 0.05 was considered significant. Kolmogorov-Smirnov test was used to determine whether the data followed the normal distribution. Comparisons between the two groups were executed by unpaired Student's *t*-test or Mann-Whitney *U*-test.

Reference

Li TL, Wang ZQ, 2011. Point mutation at the nbs1 threonine 278 site does not affect mouse development, but compromises the chk2 and smc1 phosphorylation after DNA damage. *Mechanisms of Ageing and Development*, 132(8-9):382-388. https://doi.org/10.1016/j.mad.2011.05.001