Chinese Medical Journal®

RNF115 deficiency upregulates autophagy and inhibits hepatocellular carcinoma growth

Zhaohui Gu[1], Jinqiu Feng[2], Shufang Ye[1], Tao Li[1], Yaxin Lou[3], Pengli Guo[1], Ping Lv[1], Zongming Zhang[2], Bin Zhu[4], Yingyu Chen[1,5]

[1]Department of Immunology, Peking University School of Basic Medical Sciences, NHC Key Laboratory of Medical Immunology, Peking University, Beijing 100191, China;
[2]Department of General Surgery, Beijing Electric Power Hospital, State Grid Corporation of China, Capital Medical University, Key Laboratory of Geriatrics (Hepatobiliary Diseases), China General Technology Group, Beijing 100073, China;
[3]Medical and Healthy Analytical Center, Peking University, Beijing 100191, China;
[4]Department of Orthopaedics, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China;
[5]Center for Human Disease Genomics, Peking University, Beijing 100191, China.

To the Editor: Autophagy is an evolutionarily conserved lysosome-mediated degradation process in eukaryotic cells that is crucial for maintaining cell homeostasis under normal conditions. Autophagy involves the formation of a crescent-shaped phagophore (or the isolation membrane [IM]), which expands and closes to form a double-membraned autophagosome. The autophagosome is delivered to the late endosome/lysosomes for degradation of the sequestrated materials. Autophagic dysfunction can cause tumors and neurodegenerative, cardiovascular, and autoimmune diseases.[1] Ring finger protein 115 (RNF115) is a RAB7 target protein also known as breast cancer-associated gene 2 (BCA2).[2] Accumulating studies have demonstrated that RNF115 is overexpressed in invasive breast tumors, lung adenocarcinoma, and gastric cancer. RNF115 levels were negatively correlated with prognosis in such patients. Recent studies have demonstrated that RNF115 negatively regulates phagosome maturation and the host response to bacterial infection.[3] Furthermore, *RNF115* deficiency alleviated acute liver injury in mice by promoting autophagy and inhibiting the inflammatory response.[4] However, information on the function of RNF115 in hepatocellular carcinoma remains unclear. In the present study, we report that RNF115 is a negative regulator of basal autophagy in hepatoma cells. HepG2 and HEK293T cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). BEL-7402 was obtained from the China Center for Type Culture Collection (Shanghai, China). For this study, all cell lines were authenticated using Short Tandem Repeat (STR) analysis and checked for mycoplasma-free.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000003466

All animal experimental procedures and techniques were approved by the Animal Ethics Committee of Peking University Health Sciences Center (LA2019203). The physiological effects of RNF115 on regulating autophagy were investigated using experiments involving *RNF115*-silenced hepatoma cells. We examined microtubule-associated protein 1 light chain 3 (MAP1LC3/LC3) lipidation and degradation of the autophagy substrate SQSTM1/P62, which are two widely used assays for monitoring autophagic activity. *RNF115* knockdown in HepG2 and BEL-7402 cells significantly elevated endogenous LC3B-II accumulation compared with the *sicontrol* group in basal and starved conditions [Supplementary Figure 1A, B, http://links.lww.com/CM9/C297]. In line with the Western blotting results, the *RNF115*-depleted BEL-7402 cells had increased endogenous LC3B puncta [Supplementary Figure 1C, D, http://links.lww.com/CM9/C297]. Simultaneously, *RNF115* deficiency significantly decreased SQSTM1/P62 levels [Supplementary Figure 1A, B, http://links.lww.com/CM9/C297]. Furthermore, rapamycin (RAPA) treatment facilitated SQSTM1 degradation in the *RNF115*-silenced BEL-7402 cells [Supplementary Figure 1E, F, http://links.lww.com/CM9/C297]. In the presence of bafilomycin A1 (Baf.A1), decreased SQSTM1 was blocked in the *RNF115*-silenced hepatoma cells [Supplementary Figure 1G, H, http://links.lww.com/CM9/C297], indicating that SQSTM1/P62 degradation

Zhaohui Gu, Jinqiu Feng, and Shufang Ye contributed equally to this work.

Correspondence to: Zongming Zhang, Department of General Surgery, Beijing Electric Power Hospital, State Grid Corporation of China, Capital Medical University, Beijing 100073, China
E-Mail: zhangzongming@mail.tsinghua.edu.cn;
Bin Zhu, Department of Orthopaedics, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China
E-Mail: zhubin@bjmu.edu.cn;
Yingyu Chen, Center for Human Disease Genomics, Peking University, Beijing 100191, China
E-Mail: yingyu_chen@bjmu.edu.cn

Chinese Medical Journal 2025;138(6)
Received: 18-04-2024; **Online:** 11-02-2025 **Edited by:** Xiangxiang Pan

involved the autophagy-lysosomal pathway. These results indicated that *RNF115* negatively regulated autophagic activity in the hepatoma cells.

During autophagy, the homotypic fusion and protein sorting (HOPS) complex is recruited to the lysosome membrane by binding to RAB7 and promotes autophagosome maturation.[5] Accordingly, we investigated whether RNF115-regulated autophagosome maturation was related to the HOPS complex. Co-immunoprecipitation (CO-IP) assays revealed that either FLAG-VPS39 or FLAG-VPS41 co-immunoprecipitated the overexpressed green fluorescent protein (GFP)-RNF115 and endogenous RNF115 [Supplementary Figure 2A–D, http://links.lww.com/CM9/C297]. The RNF115 protein contains an N-terminal ubiquitin-binding zinc finger (BCA2 zinc finger [BZF]) domain, a protein kinase B (AKT) phosphorylation domain, and a C-terminal RING-H2 domain [Supplementary Figure 2E, http://links.lww.com/CM9/C297]. Therefore, we delineated the RNF115 domains that bind to VPS39 or VPS41. We determined that GFP-VPS39 or -VPS41 co-precipitated the 101–200 amino acid region of RNF115, whereas the RNF115 N- and C-terminals exhibited no interactions [Supplementary Figure 2F, G, http://links.lww.com/CM9/C297]. Next, we determined the biological significance of the RNF115–HOPS complex interaction. Knocking down *RNF115* increased the VPS39 and VPS41 protein expression levels in an obvious manner [Supplementary Figure 3A–D, http://links.lww.com/CM9/C297]. Conversely, overexpressing *RNF115* reduced the expresssion levels of two proteins [Supplementary Figure 3E–H, http://links.lww.com/CM9/C297]. Subsequently, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) testing of the *VPS39* and *VPS41* transcript levels revealed that knocking down *RNF115* increased the *VPS39* and *VPS41* messenger RNA (mRNA) levels [Supplementary Figure 3I–K, http://links.lww.com/CM9/C297]. Contrastingly, *RNF115* overexpression decreased the *VPS39* and *VPS41* mRNA levels [Supplementary Figure 3L–N, http://links.lww.com/CM9/C297]. Furthermore, we tested whether the VPS39/VPS41 reduction in the *RNF115*-overexpressing cells could be attributed to increased proteasomal or lysosomal degradation. Supplementary Figure 3O, http://links.lww.com/CM9/C297 demonstrates that the decreased VPS39 did not recover in the MG132-treated cells, while MG132 largely restored VPS41 expression in the *RNF115*-overexpressing cells [Supplementary Figure 3P, http://links.lww.com/CM9/C297]. These data suggested that RNF115 negatively regulated VPS39/VPS41 expression in transcription and post-translational modification.

The HOPS complex and the GTPase RAB7 are critical for tethering autophagosomes with lysosomes, followed by fusion of the membrane bilayer. We demonstrated that RNF115 interacts with RAB7 [Supplementary Figure 4A, http://links.lww.com/CM9/C297]. As RNF115 interacts with RAB7, VPS39, and VPS41, we examined whether RNF115 could affect the RAB7–VPS39–VPS41 interaction. We determined that the *RNF115*-depleted cells had significantly increased amounts of RAB7-precipitated VPS39/VPS41 compared with the control cells [Supplementary Figure 4B, C, http://links.lww.com/CM9/C297]. These observations demonstrated that RNF115 negatively regulated the HOPS complex and the binding affinity of RAB7 with the HOPS complex, thereby decreasing autophagosome-lysosome fusion.

Autophagy related 14 (ATG14) is localized in the endoplasmic reticulum and is a component of the phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3) complex, which promotes autophagosome formation. ATG14 also binds to syntaxin 17 (STX17) and increases STX17–synaptosome associated protein 29 (SNAP29)–vesicle associated membrane protein 8 (VAMP8) complex assembly, promoting autophagosome-lysosome fusion.[6] Our results delineated the ability of RNF115 to interact with ATG14 with or without Earle's balanced salt solution (EBSS) incubation [Supplementary Figure 5A, http://links.lww.com/CM9/C297]. Overexpressing *RNF115* decreased GFP-ATG14 levels [Supplementary Figure 5B, C, http://links.lww.com/CM9/C297], whereas silencing *RNF115* increased ATG14 protein expression [Supplementary Figure 5D, E, http://links.lww.com/CM9/C297]. Further investigation proved that MG132 treatment of the RNF115-overexpressing cells could recover the decreased ATG14 [Supplementary Figure 5F, http://links.lww.com/CM9/C297], indicating that the E3 ubiquitin ligase RNF115 downregulated ATG14 through the proteasome pathway. Additionally, RNF115 negatively regulated the *ATG14* transcript levels [Supplementary Figure 5G, H, http://links.lww.com/CM9/C297]. These data suggested that RNF115 negatively regulated ATG14 expression in transcription and post-translational modification.

The function of RNF115 in hepatocellular carcinoma development was explored by screening stable *RNF115* knockdown HepG2 and BEL-7402 cell lines using *HBLV-shRNF115* lentiviral vectors. We observed that the stable *RNF115* knockdown cells had decreased SQSTM1 levels and accumulated LC3B-II [Supplementary Figure 6A, B, http://links.lww.com/CM9/C297], indicating the increased autophagic activity in the cells. The cell viability and colony formation assays demonstrated that knocking down *RNF115* blocked hepatoma cell growth [Supplementary Figure 6C–F, http://links.lww.com/CM9/C297]. Furthermore, the wound healing [Supplementary Figure 6G and 6H, http://links.lww.com/CM9/C297] and Transwell assays [Supplementary Figure 6I–L, http://links.lww.com/CM9/C297] demonstrated that silencing *RNF115* delayed hepatoma cell migration.

We also investigated whether RNF115 affects the biological behavior of hepatocellular carcinoma cells *in vivo* using a nude mouse tumorigenicity assay. HBLV-*shcontrol* BEL-7402 cells and HBLV-*shRNF115* BEL-7402 cells (2.5×10^6 cells) were subcutaneously injected into each BALB/c nude mouse. The results demonstrated that knocking down *RNF115* significantly inhibited BEL-7402 cell growth in the mice compared with the control group [Supplementary Figure7A–C, http://links.lww.com/CM9/C297]. The role of RNF115 in hepatocellular carcinoma was futher studied by generating *Rnf115* knockout (KO) mice,[4] and 14-day-old male *Rnf115*$^{-/-}$ mice and wild-type (WT) C57BL/6 mice received a single intraperitoneal injection of diethylnitrosamine (DEN). The mouse body

weights were examined within 11 months after DEN administration [Supplementary Figure 7D, http://links.lww.com/CM9/C297]. Our results demonstrated that the *Rnf115*$^{-/-}$ mice developed fewer tumors than the WT mice [Supplementary Figure 7E, F, http://links.lww.com/CM9/C297]. The hematoxylin–eosin (H&E) staining suggested that the DEN-induced *Rnf115*$^{+/+}$ livers contained more irregular tumor cell clusters than the *Rnf115*$^{-/-}$ liver tissues [Supplementary Figure 7G, http://links.lww.com/CM9/C297], indicating that *Rnf115* deficiency inhibited hepatocellular carcinoma development. Next, we performed an immunofluorescence assay and determined that the DEN-induced *Rnf115*$^{+/+}$ livers had a significantly higher fluorescence signal of SQSTM1 protein than the *Rnf115*$^{-/-}$ liver tissues [Supplementary Figure 8, http://links.lww.com/CM9/C297], suggesting that the decreased SQSTM1 accumulation in the *Rnf115*$^{-/-}$ liver contributed to the inhibition of tumor progression. Collectively, the *in vivo* and *in vitro* results demonstrated that RNF115-mediated autophagy was negatively correlated with hepatocellular carcinoma occurrence and development.

In summary, we demonstrated that RNF115 interacts with the HOPS complex and ATG14, negatively regulating their expression. Inactivating *RNF115* promoted autophagic activity by enhancing RAB7–HOPS complex binding and ATG14 activities [Figure 1], which inhibited hepatoma cell growth *in vivo* and *in vitro* and provided an experimental foundation for hepatocellular carcinoma treatment by targeting RNF115.

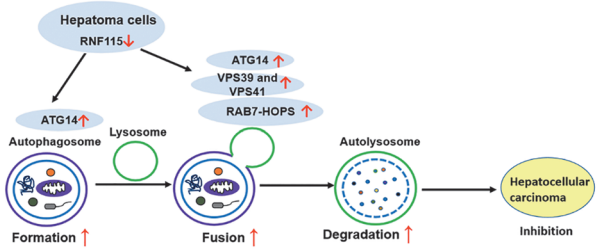

Figure 1: RNF115 negatively regulates autophagy and promotes hepatocellular carcinoma growth. Inactivation of *RNF115* in hepatoma cells increases the expression of ATG14, VPS39, and VPS41, promotes RAB7-HOPS complex binding, and finally inhibits the growth of hepatoma cell by upregulating autophagic activities. ATG14: Autophagy related 14; HOPS: Homotypic fusion and protein sorting; VSP: Vacuolar protein sorting; RAB: Ras-associated protein; RNF115: Ring finger protein 115.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 91954116), China Postdoctoral Science Foundation (No. 2023M730128), the Key Support Project of Guo Zhong Health Care of China General Technology Group (No. GZKJ-KJXX-QTHT-20230626).

Conflicts of interest

None.

References

1. Yamamoto H, Zhang S, Mizushima N. Autophagy genes in biology and disease. Nat Rev Genet 2023;24:382–400. doi: 10.1038/s41576-022-00562-w.
2. Mizuno K, Kitamura A, Sasak T. Rabring7, a novel Rab7 target protein with a RING finger motif. Mol Biol Cell 2003;14:3741–3752. doi: 10.1091/mbc.e02-08-0495.
3. Bilkei-Gorzo O, Heunis T, Marín-Rubio JL, Cianfanelli FR, Raymond BBA, Inns J, *et al*. The E3 ubiquitin ligase RNF115 regulates phagosome maturation and host response to bacterial infection. EMBO J 2022;41:e108970. doi: 10.15252/embj.2021108970.
4. Feng J, Ye S, Hai B, Lou Y, Duan M, Guo P, *et al*. RNF115/BCA2 deficiency alleviated acute liver injury in mice by promoting autophagy and inhibiting inflammatory response. Cell Death Dis 2023;14:855. doi: 10.1038/s41419-023-06379-7.
5. Jiang P, Nishimura T, Sakamaki Y, Itakura E, Hatta T, Natsume T, *et al*. The HOPS complex mediates autophagosome-lysosome fusion through interaction with syntaxin 17. Mol Biol Cell 2014;25:1327–1337. doi: 10.1091/mbc.e13-08-0447.
6. Liu R, Zhi X, Zhong Q. ATG14 controls SNARE-mediated autophagosome fusion with a lysosome. Autophagy 2015;11:847–849. doi: 10.1080/15548627.2015.1037549.

How to cite this article: Gu ZH, Feng JQ, Ye SF, Li T, Lou YX, Guo PL, Lv P, Zhang ZM, Zhu B, Chen YY. *RNF115* deficiency upregulates autophagy and inhibits hepatocellular carcinoma growth. Chin Med J 2025;138:754–756. doi: 10.1097/CM9.0000000000003466