COMMENTARY

COMMENTARY

Linking a nuclear lncRNA to cytoplasmic lysosome integrity and cell death

Haibin Liu[a] and Zhi-Ming Zheng[a,1]

Long noncoding RNAs (lncRNAs) are noncoding RNAs that are ≥200 nucleotides in size and may function in diverse biological processes (1). Although the human genome may encode up to ∼100,000 lncRNAs, most lncRNAs are at low abundance and it is hard to tell their real function. However, a subset of highly expressed lncRNAs are regulatory RNAs with profound functions in the regulation of gene transcription, paraspeckle formation, RNA splicing, messenger RNA (mRNA) stability, and protein degradation (1–3). Most lncRNAs are transcribed by RNA polymerase II and their expression is regulated at both transcription and RNA processing levels (1) and by various virus infections (4, 5). In PNAS, Yang et al. (6) explore the expression profile of cellular lncRNAs in the presence of the histone deacetylase inhibitor Trichostatin A and identify a ∼2-kb-long nuclear lncRNA, ENSG00000273148 or LINC00653, highly expressed from chr20 in p53-null, non–small-cell lung cancer cell line NCI-H1299 cells. Yang et al. (6) show that it regulates lysosomal-associated protein transmembrane 5 (LAPTM5) expression and lysosome cell death (LCD); the authors thus name this lncRNA an LCD regulator, or LCDR.

Lysosomes are small cytoplasmic and acidic membrane-bound organelles and contain over 50 hydrolytic enzymes (7) for the digestion of various extra- and intracellular materials to maintain cell homeostasis and destroy invading pathogens. Among ∼25 integral lysosomal membrane proteins (8), LAPTM5, which was thought to be specifically expressed in hematopoietic cells (9) to maintain lysosome membrane integrity (8), is also highly expressed in human bladder cancer tissues. Knockdown (KD) of LAPTM5 expression in bladder cancer cell lines T24 and 5637 inhibits cell proliferation and colony formation (10), but the mechanism of LAPTM5 in relation to cell growth function remains unknown. Thus, Yang et al. (6) move one step closer to understanding how LAPTM5 promotes lysosomal membrane integrity and contributes to cancer cell survival, a finding that links LCDR and LAPTM5 as responsible for cytoplasmic lysosomal biogenesis and function.

LAPTM5 complementary DNA (cDNA) was cloned in 1996 (9). The *LAPTM5* gene on chr1p34 encodes a pre-mRNA containing eight exons, which is processed by nuclear RNA splicing to produce a ∼2.6-kb mRNA and, after RNA export, encode a ∼29-kDa LAPTM5 protein preferentially localized to the lysosome membrane (9, 11) along with other lysosomal proteins, such as LAMP1/2 (8) (Fig. 1). Yang et al. (6) show that among six lysosomal proteins (ACP2, ARSB, ASAH1, GLB1, HGSNAT, and LAPTM5) susceptible to up-regulation by both LCDR and heterogenous nuclear ribonucleoprotein K (hnRNPK), short interfering RNA (siRNA)–mediated reduction of LAPTM5 expression turned out to be the only one causing lysosomal membrane permeabilization (LMP), cell apoptosis, and decreased cell proliferation and colony formation (6). Although all of these results are consistent with the data by KD of LCDR or hnRNPK expression to reduce LAPTM5 expression in NCI-H1299 cells, the observed LAPTM5 function in the regulation of the programmed cell death that was unrelated to other apoptosis-causing genes in Yang et al. (6) is interesting. But the data appear to contradict studies where overexpression of LAPTM5 in neuroblastoma cells was found to cause LMP and nonapoptotic cell death (12) and in HeLa cells to induce cleavage of Mcl-1, Bid, caspases, and PARP, leading to mitochondria-dependent apoptosis (13). How LAPTM5 both at an increased or decreased level induces cell apoptosis and cell death in a cell-type–dependent manner remains unknown. Obviously, this contradictory, but interesting, result and some fundamental questions need to be carefully investigated.

In the investigation of how nuclear LCDR functions in NCI-H1299 cells to maintain LAPTM5

[a]Tumor Virus RNA Biology Section, HIV Dynamics and Replication Program, National Cancer Institute, NIH, Frederick, MD 21702

Author contributions: H.L. and Z.-M.Z. wrote the paper.

The authors declare no competing interest.

This article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

See companion article, "*LCDR* regulates the integrity of lysosomal membrane by hnRNP K–stabilized *LAPTM5* transcript and promotes cell survival," 10.1073/pnas.2110428119.

[1]To whom correspondence may be addressed. Email: zhengt@exchange.nih.gov.

Published February 15, 2022.

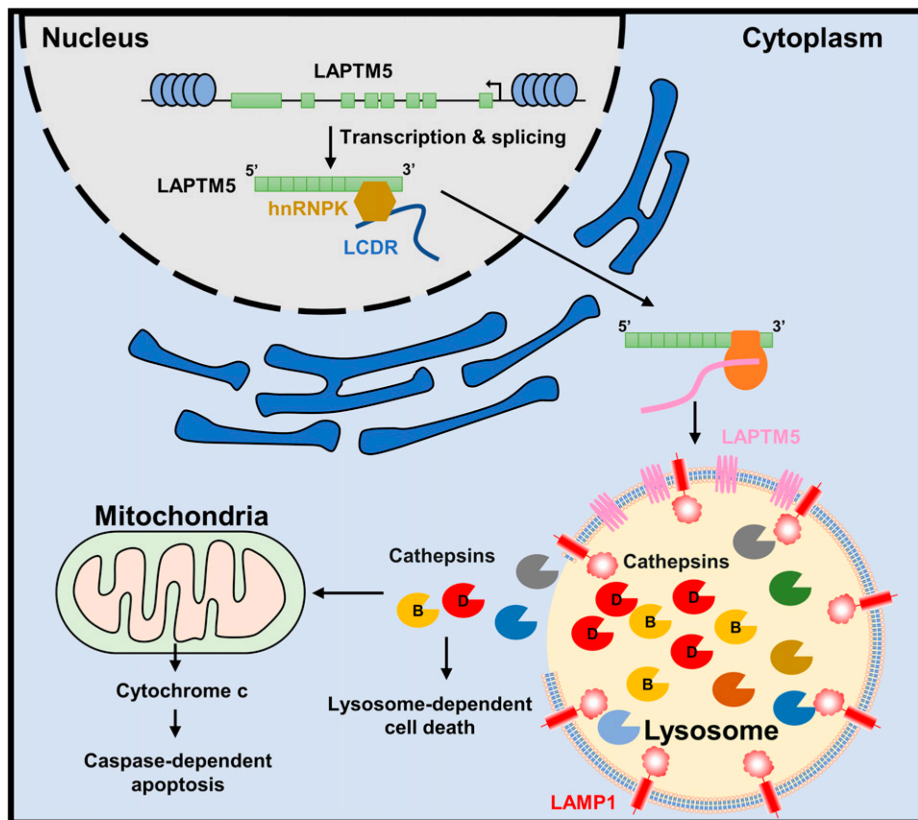

Fig. 1. LCDR lncRNA promotes LAPTM5 expression by stabilizing nuclear LAPTM5 RNA through hnRNPK. LAPTM5 protein facilitates lysosomal membrane integrity and thus prevents LMP, which can be triggered by different inducers. Reduction of LAPTM5 expression leads to damage of the lysosome membrane and thus LMP to the release of cathepsins B and D and other hydrolases to the cytosol, causing lysosome-dependent cell death. Released cathepsins also cause other types of cell death, including caspase-dependent apoptosis by promoting mitochondrial outer membrane permeabilization and release of cytochrome *c* to activate programmed cell death by activated caspases (19, 20).

expression, lysosome membrane integrity, cell proliferation, colony formation, and xenograft tumorigenesis, Yang et al. (6) identify a multifunctional RNA-binding protein (RBP), hnRNPK (14), which specifically interacts with both LCDR and LAPTM5 RNA transcripts. As an abundant nuclear RBP, hnRNPK, like other hnRNPs, plays many roles in RNA transcription, splicing, stability, transport, and translation despite the fact that each hnRNP may have a distinct set of RNA targets to specify its biological function (14, 15). hnRNPK contains four RNA binding domains, three K-Homology (KH) domains, and an arginine–glycine (RGG)-rich domain, for interaction with an RNA single-stranded poly(C) region (16). Yang et al. reveal that the 5′ end of LCDR bears a poly(C) site to bind the KH1 domain of hnRNPK and the 3′ end of LAPTM5 RNA has a poly(C) site to bind the KH3 domain of hnRNPK. Although hnRNPK binding to the LAPTM5 RNA could stabilize nuclear LAPTM5 RNA and enhance expression of LAPTM5 protein, Yang et al. find that hnRNPK binding to LCDR RNA does not affect LCDR expression, nor does KD of LCDR expression affect the hnRNPK level. However, LCDR expression was found to be essential for LAPTM5 protein expression, possibly by enhancing hnRNPK interaction with LAPTM5 RNA (Fig. 1). Thereby, KD expression of LCDR in the NCI-H1299 cells, as seen for KD hnRNPK, led to the instability of LAPTM5 RNA, reduction of the LAPTM5 protein level, and an increase of LMP and LCD (6). These groundbreaking observations clearly point out that hnRNPK serves as a mediator to guide LCDR in interaction with and stabilization of LAPTM5 RNA, although further mechanistic studies are needed.

Given that LCDR, hnRNPK, and LAPTM5 all highly express in lung cancer tissues and reduction of the expression of any one of the three leads to LMP and LCD, Yang et al. (6) establish a lung cancer patient–derived xenograft (PDX) mouse model and examine nuclear targeting nanoparticle (NT-NP)–mediated intravenous delivery of LCDR-specific siRNAs (NT-NPs si-LCDR) for treatment of PDX mouse tumor. The treatment regimen was 1 nmol NT-NPs si-LCDR per injection per mouse every 2 d for three consecutive doses. The primary data from this biomarker-targeted therapy were astonishing by 18 d after PDX. The authors show that the intravenously injected NT-NPs si-LCDR, which was preferentially accumulated in tumor tissues, strongly inhibited tumor growth in all five PDX mice with remarkably reduced expression of LCDR and LAPTM5 and a significantly increased level of cleaved caspase 3 in the tumor tissues, when compared with the mice receiving phosphate-buffered saline or control NT-NPs treatment. Despite recent application of PD-1/PD-L1 inhibitors to improve the efficiency of the treatment of lung cancer (17), Yang et al.'s NT-NPs si-LCDR approach and the promising results from their lung cancer PDX mouse tumor model highlight an important step in the development of a biomarker-targeted therapy and set forth further preclinical and clinical trials for possible treatment of human cancer.

With the recent focus on lncRNAs and lncRNA functions, Yang et al. (6) present a series of attractive results of this nuclear

lncRNA LCDR in the regulation of lysosomal membrane integrity and cancer cell survival. It would be timely to understand in more detail the described LCDR/hnRNP K/LAPTM5 axis in cell biology and carcinogenesis. As an abundant nuclear lncRNA, LCDR must be multifunctional, hnRNPK must not be the only RBP to LCDR, and LAPTM5 must not be the only target of LCDR. How to prioritize each of the remaining questions will be challenging to address. Whether LCDR can be developed as a predictive biomarker for cancer diagnosis and targeted cancer therapy will be an ultimate goal long to reach. Together with a recent report on LAPTM5 restriction of HIV infection in macrophages (18), the paradigm-shifting findings by Yang et al. provide an excellent example bridging a nuclear lncRNA to cytoplasmic control of lysosome integrity and metabolism for cell homeostasis and antipathogen defense, a next frontier for the lncRNA field.

Acknowledgments

The Z.M.Z. laboratory was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research (ZIASC010357).

1 L. Statello, C. J. Guo, L. L. Chen, M. Huarte, Gene regulation by long non-coding RNAs and its biological functions. *Nat. Rev. Mol. Cell Biol.* **22**, 96–118 (2021).
2 M. Aznaourova *et al.*, Noncoding RNA *MaIL1* is an integral component of the TLR4-TRIF pathway. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 9042–9053 (2020).
3 C. J. Guo *et al.*, Distinct processing of lncRNAs contributes to non-conserved functions in stem cells. *Cell* **181**, 621–636.e22 (2020).
4 Q. Zhang, C. Y. Chen, V. S. Yedavalli, K. T. Jeang, NEAT1 long noncoding RNA and paraspeckle bodies modulate HIV-1 posttranscriptional expression. *MBio* **4**, e00596–e12 (2013).
5 H. Liu *et al.*, Oncogenic HPV promotes the expression of the long noncoding RNA lnc-FANCI-2 through E7 and YY1. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2014195118 (2021).
6 X. Yang *et al.*, *LCDR* regulates the integrity of lysosomal membrane by hnRNP K–stabilized *LAPTM5* transcript and promotes cell survival. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2110428119 (2022).
7 M. Schwake, B. Schröder, P. Saftig, Lysosomal membrane proteins and their central role in physiology. *Traffic* **14**, 739–748 (2013).
8 P. Saftig, J. Klumperman, Lysosome biogenesis and lysosomal membrane proteins: Trafficking meets function. *Nat. Rev. Mol. Cell Biol.* **10**, 623–635 (2009).
9 C. N. Adra *et al.*, LAPTM5: A novel lysosomal-associated multispanning membrane protein preferentially expressed in hematopoietic cells. *Genomics* **35**, 328–337 (1996).
10 L. Chen *et al.*, Downregulation of LAPTM5 suppresses cell proliferation and viability inducing cell cycle arrest at G0/G1 phase of bladder cancer cells. *Int. J. Oncol.* **50**, 263–271 (2017).
11 Y. Pak, W. K. Glowacka, M. C. Bruce, N. Pham, D. Rotin, Transport of LAPTM5 to lysosomes requires association with the ubiquitin ligase Nedd4, but not LAPTM5 ubiquitination. *J. Cell Biol.* **175**, 631–645 (2006).
12 J. Inoue *et al.*, Lysosomal-associated protein multispanning transmembrane 5 gene (LAPTM5) is associated with spontaneous regression of neuroblastomas. *PLoS One* **4**, e7099 (2009).
13 D. Y. Jun *et al.*, Ectopic overexpression of LAPTM5 results in lysosomal targeting and induces Mcl-1 down-regulation, Bak activation, and mitochondria-dependent apoptosis in human HeLa cells. *PLoS One* **12**, e0176544 (2017).
14 Z. Wang *et al.*, The emerging roles of hnRNPK. *J. Cell. Physiol.* **235**, 1995–2008 (2020).
15 T. Geuens, D. Bouhy, V. Timmerman, The hnRNP family: Insights into their role in health and disease. *Hum. Genet.* **135**, 851–867 (2016).
16 P. H. Backe, A. C. Messias, R. B. Ravelli, M. Sattler, S. Cusack, X-ray crystallographic and NMR studies of the third KH domain of hnRNP K in complex with single-stranded nucleic acids. *Structure* **13**, 1055–1067 (2005).
17 K. C. Arbour, G. J. Riely, Systemic therapy for locally advanced and metastatic non-small cell lung cancer: A review. *JAMA* **322**, 764–774 (2019).
18 L. Zhao *et al.*, Vpr counteracts the restriction of LAPTM5 to promote HIV-1 infection in macrophages. *Nat. Commun.* **12**, 3691 (2021).
19 P. Boya, G. Kroemer, Lysosomal membrane permeabilization in cell death. *Oncogene* **27**, 6434–6451 (2008).
20 F. Wang, R. Gómez-Sintes, P. Boya, Lysosomal membrane permeabilization and cell death. *Traffic* **19**, 918–931 (2018).