RESEARCH HIGHLIGHT

LETN and NPM1 tango in human nucleoliGuang Xu¹, Man Wu¹ and Ling-Ling Chen^{1,2,3} *Cell Research* (2021) 31:609–610; <https://doi.org/10.1038/s41422-021-00471-3>

In a recent paper published in *Cell Research*, Wang et al. identify a nucleolar-localized long noncoding RNA (lncRNA), *LETN* (lncRNA essential for tumor cell proliferation via NPM1) and depict a mutually dependent NPM1–*LETN* interaction that is essential for maintaining nucleolar function and sustaining cancer cell proliferation.

RNAs are engaged with proteins during their life cycles, so are long noncoding RNAs (lncRNAs). Due to their large sizes and dynamic conformations, functional lncRNAs interact with different sets of proteins to ensure their proper processing and subcellular localizations. However, functions of proteins are generally thought to be independent of RNAs. In a recent study in *Cell Research*, Wang et al.¹ uncovered that lncRNA *LETN* is required for the protein NPM1 to execute its multiple roles.

LETN is a polyadenylated, primate-specific lncRNA with ~4500 nt in length (Fig. 1a). Its expression is low in most normal human tissues, but is upregulated in embryonic tissues and multiple types of tumor. *LETN* is largely localized in the nucleolus and is critical for cancer cell proliferation and tumor development. Knockdown or overexpression of *LETN* reduced or accelerated tumor cell proliferation, respectively. Mainly responsible for rRNA biogenesis, the nucleolus is the biggest nuclear condensate assembled via liquid–liquid phase separation (LLPS) and composed of three morphologically distinct sub-compartments: fibrillar center (FC), dense fibrillar component (DFC) and granular component (GC) regions.² Prior work has suggested that lncRNAs are involved in ribosome biogenesis, e.g., *SLERT* is localized to FC/DFC regions and promotes polymerase I (Pol I) transcription.³ Wang et al. showed that *LETN* was mainly enriched in GC regions and that *LETN* depletion led to GC distortion without affecting FC/DFCs.¹

GC distribution of *LETN* relies on direct interaction with the GC protein NPM1, which functions as pentamers in nucleolar structures,^{4,5} ribosome biogenesis and chromatin remodeling.⁶ The authors found that *LETN* interacted with NPM1 via the C-terminal domain containing the nucleolar localization signal (NoLS) (Fig. 1a). Repression of NPM1 expression resulted in similar phenotypes as seen by loss of *LETN*: decreased cell proliferation, disturbed nucleoli, suppressed chromatin compaction and reduced Pol I transcription, indicating that NPM1 requires *LETN* for functional activation. It is worth noting that an even GC distribution of NPM1 in live cells vs a ring-like GC distribution in fixed cells were observed.¹ It will be of interest to clarify these distinct NPM1 subcellular distribution patterns.

The authors next performed multiple assays to examine a mutual dependent role of *LETN* and NPM1. First, they dissected the RNA–protein interaction in detail. On one hand, NPM1 bound to three regions in *LETN*, 180 nt in length for each. Deleting all three regions completely blocked the *LETN*–NPM1 interaction. Re-

introducing the first 2000 nt of *LETN* containing these regions, but not a simple combination of the three fragments, partially rescued cell proliferation. On the other hand, *LETN* promoted NPM1 pentamerization via its N-terminal domain both in vitro and in cells (Fig. 1a). Consequently, phenotypes related to pentamerized NPM1, such as the interaction between NPM1 and histone proteins⁷ as well as nucleolar morphology⁶ were found to be impaired upon *LETN* depletion.

Next, the authors showed that *LETN* nucleolar localization was dependent on the full-length NPM1 (NPM1-F); meanwhile, *LETN* carried out its function mainly via NPM1 (Fig. 1b). *LETN* overexpression/knockdown enhanced/suppressed cell proliferation and chromatin condensation only in NPM1-F-expressing cells but not in cells expressing N- or C-terminally truncated NPM1 (NPM1-ΔN/ΔC).¹ Mechanistically, NPM1-ΔN failed to form oligomers whereas NPM1-ΔC failed to recruit *LETN* to nucleoli. Thus, the interaction between *LETN* and NPM1, rather than each factor alone, is necessary for their functions. However, how the interaction between the C-terminal fragment of NPM1 and *LETN* affects NPM1 pentamerization at the N-terminus is warranted for future study.

The authors also examined whether the regulation of ribosome biogenesis by NPM1–*LETN* interaction is physiologically relevant in cancer context, as hyperactive rRNA production is related to fast cell proliferation.⁸ As expected, liver cancer patients with better prognosis displayed lower levels of NPM1 and *LETN*, whereas patients with worse prognosis exhibited higher levels of both factors. Such a reverse correlation thus supports the view that the NPM1–*LETN* interaction in ribosome biogenesis is associated with cancer progression.

Finally, the mutual dependency between NPM1 and *LETN* was supported from an evolutionary point of view. While NPM1 is highly conserved across species, *LETN* is only present in primates (Fig. 1c). In NPM1-depleted human HUH7 cells, re-introducing either mouse or human NPM1 (mNPM1 or hNPM1) largely restored cell proliferation and rRNA biogenesis. Intriguingly, the rescue effects of hNPM1 are dependent on *LETN*, but those of mNPM1 are not. Further, *LETN* was required for pentamerization of hNPM1 but not mNPM1. It turned out that hNPM1 C-terminal region harbors four different residues from mNPM1, among which two Ser residues play a key role in the NPM1–*LETN* interaction (Fig. 1a–c). The hNPM1-214A/217A mutant lost its capabilities of pentamerization or binding with *LETN*. In an elegant reciprocal set of experiments, conversion of mNPM1 to its human homolog at these two residues (mNPM1-214S/217S) largely reactivated its binding with *LETN*, as well as its pentamerization. These results together suggest that the two Ser residues of hNPM1 are key to NPM1–*LETN* function in human cells.

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Fig. 1 *LETN* and NPM1 are mutually dependent for nucleolar functions in primates. **a** *LETN* is a primate-specific, nucleolar-localized lncRNA that binds the C-terminal region of NPM1 to facilitate NPM1 pentamerization at the N-terminal domain with an unknown mechanism. **b** *LETN* promotes human cancer cell proliferation by driving main functions of NPM1, such as maintaining rRNA biogenesis and supporting GC integrity of the nucleolus. **c** Two newly evolved Ser residues of human NPM1 are essential for the NPM1–*LETN* function in human cells, whereas mouse NPM1 that lacks these two residues functions independently of *LETN*, which is absent in rodents.

Collectively, the human-specific protein–lncRNA interaction presents an intriguing regulation for acquiring a hyperactive nucleolus in fast cell proliferation relevant to cancers. The mutual requirement of hNPM1 and *LETN* raises the notion that this newly emerged lncRNA in evolution is indispensable for hNPM1's major functions. Similarly, a recent study suggests that lncRNA orthologs undergo distinct species-specific processing for primate-specific functions.⁹ Thus, the prevalent expression and processing of lncRNAs in primates appears to be more meaningful in gaining primate-specific roles than previously thought. Additionally, it also raises some questions that need to be further addressed. For instance, how could ~200 copies of *LETN* per cell adequately modulate ~8,800,000 copies of NPM1?¹⁰ Do the other two of the four different residues between hNPM1 and mNPM1 play any role in NPM1's function? How does *LETN* arise during evolution, and whether it is common to other primate-specific lncRNAs? Do changes in NPM1 residues co-evolve with *LETN* emergence? Given the species-specific expression of lncRNAs, it is possible that mutually independent protein–lncRNA pairs may be present in

lower organisms but absent in primates. Nonetheless, the current study provides clear evidence that an evolutionarily non-conserved lncRNA is indispensable for NPM1 functions in human nucleoli. Further studies of additional protein–lncRNA pairs will certainly broaden our understanding of lncRNA functions and action modes of previously characterized proteins.

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