METTL3-mediated m6A modification stabilizes TERRA and maintains telomere stability

Liping Chen, Canfeng Zhang, Wenbin Ma, Junjiu Huang, Yong Zhao, Haiying Liu

Nucleic Acids Research, *2022, Vol. 50*, No. 20 11619–11634

Speaker：Pei-Chun Chen, Advisor：Chun-Ying Yu, Date：2023.03.10

1. 簡述論文的概要和重大發現

Telomere 是位於染色體末端，用來保護其不被識別為 DNA damage，而 Telomeric repeat-containing RNA (TERRA)是一種由 telomere transcribe 出的 long noncoding RNA，會通過侵入 telomere DNA 中形成 R-loop，過多或不足的 R-loop 都會導致 telomere 不穩定，因此對於 TERRA level 需要精細的調節。

在這個研究中，作者發現 m6A modification 存在於 TERRA 的 subtelomeric region，並且可以穩定 TERRA，其機制是 METTL3 催化 TERRA 上的 m6A modification，並且由 YTHDC1 來識別並且穩定，因此 knockdown METTL3 和 YTHDC1 會增加 TERRA 的 degradation。另外，m6A modification 的 TERRA 會形成 R-loop，並且促進 homologous recombination，而這對於癌細胞的 Alternative lengthening of telomeres (ALT) pathway 至關重要，因此 knockdown METTL3 會導致 R-loop 的減少，telomere 縮短或不穩定。

總之，這些發現可以顯示 METTL3 通過催化 TERRA 上的 m6A modification 保護 telomere，並且顯示抑制或刪除 METTL3 可能是 ALT cancer 治療的新途徑。

2. 對論文內容的提問

為什麼只有 subtelomeric region 會被 m6A modification，而 telomere 沒有被 m6A modification 的原因是什麼?

Figure2 的圖 I 為什麼要用同個 sgRNA 做 ALKBH5-WT，而不是兩個 sgRNA 都做?

3. 論文的缺點與評論

這篇論文發現了 m6A modification 在維持 telomere 的穩定性中發揮了重要的作用，並且 METTL3 和 YTHDC1 可以調節 TERRA 的 m6A modification 和 TERRA 的穩定性，同時 m6A modification 的 TERRA 可以促進 homologous recombination 和 cancer cell 中的 ALT pathway，這些發現為 ALT cancer 的治療開拓了新的方向。

Published online 18 November 2022 *Nucleic Acids Research, 2022, Vol. 50, No. 20* ***11619–11634***
https://doi.org/10.1093/nar/gkac1027

METTL3-mediated m^{6}A modification stabilizes TERRA and maintains telomere stability

Liping Chen[1,2,†], Canfeng Zhang[3,†], Wenbin Ma[1], Junjiu Huang[1], Yong Zhao[1,‡] and Haiying Liu[1,*]

[1]MOE Key Laboratory of Gene Function and Regulation, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou 510006, China, [2]The Center for Medical Research, The First People's Hospital of Nanning City, Nanning 530021, China and [3]Scientific Research Center, The Seventh Affiliated Hospital, Sun Yat-Sen University, Shenzhen, 518107, China

Received January 24, 2022; Revised September 23, 2022; Editorial Decision October 16, 2022; Accepted October 21, 2022

ABSTRACT

Telomeric repeat-containing RNA (TERRA) is a type of long non-coding RNA transcribed from telomeres, and it forms R-loops by invasion into telomeric DNA. Since either an excessive or inadequate number of R-loops leads to telomere instability, the TERRA levels need to be delicately modulated. In this study, we found that m^{6}A modification presents on the subtelomeric regions of TERRA and stabilizes it, and the loss of METTL3 impacts telomere stability. Mechanically, the m^{6}A modification on TERRA is catalyzed by METTL3, recognized and stabilized by the m^{6}A reader YTHDC1. Knockdown of either METTL3 or YTHDC1 enhances TERRA degradation. The m^{6}A-modified TERRA forms R-loops and promotes homologous recombination which is essential for the alternative lengthening of telomeres (ALT) pathway in cancer cells. METTL3 depletion leads to R-loop reduction, telomere shortening and instability. Altogether, these findings reveal that METTL3 protects telomeres by catalyzing m^{6}A modification on TERRA, indicating that inhibition or deletion of METTL3 is potentially a new avenue for ALT cancer therapy.

INTRODUCTION

Telomeres are composed of tandem repeats of the DNA sequence TTAGGG/AATCCC and are located at the physical ends of eukaryotic linear chromosomes, safeguarding the natural DNA ends from being recognized as DNA damage (1–3). It is reported that 85–90% of cancer cells maintain telomere length by telomerase, while the remaining 10–15% elongate telomeres by the alternative lengthening of telomeres (ALT) pathway that utilizes homologous recombination (HR) resulting in telomeric sister chromatin exchange (T-SCE) (4–6). Although the proportion of ALT cancers is lower than that of telomerase-positive cancers, most of the ALT cancers are more malignant and the prognoses are poor. There are lots of special characteristics in ALT cancer cells such as the C-circle, ALT-associated PML bodies (APBs), heterogeneous telomere length, absence of ATRX, telomere recombination and a high telomeric repeat-containing RNA (TERRA) level (7–11). It has been reported that TERRA is required for maintenance of telomere and chromosome stability. Depletion of TERRA transcripts lead to the activation of DNA damage response at chromosome ends, resulting in telomere dysfunction-induced foci (TIFs) (12–14), indicating that TERRA is a potential target for ALT cancer therapy.

TERRA is a type of long non-coding RNA transcribed from subtelomeric regions to telomeres by RNA polymerase II (Pol II), with heterogeneous length ranging from 100 bp to 9 kb (8,9). It has been reported that TERRA can bind to telomeres and form R-loops (the three-stranded nucleic acid structures that consist of an RNA–DNA hybrid and a displaced DNA strand) both in yeast and in human cells (15–20). The R-loops formed by TERRA may act as a double-edged sword in telomere length maintenance (21). Rajika Arora *et al.* revealed that depletion of RNase HI results in telomeric R-loop accumulation, which induces replication stress and telomere shortening (20). In contrast, overexpression of RNase HI causes a decrease in HR and telomere shortening in recombination-competent yeast and ALT cells, indicating that TERRA-formed R-loops play a physiological role at telomeres (16,20). The double-faced roles of TERRA in telomere length maintenance raises the hypothesis that TERRA expression should be tightly regulated to maintain appropriate levels required for ALT.

N^6-Methyladenosine (m^{6}A) is one of the most prevalent and reversible internal RNA modifications among numer-

*To whom correspondence should be addressed. Email: liuhy5@mail.sysu.edu.cn
†The authors wish it to be known that, in their opinion, the first two authors should be regarded as Joint First Authors.
‡Deceased April 8, 2021.