School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

Title of Doctor Dissertation:

Characterization of tRNA Methyltransferase 2 Homolog A, TRMT2A, in Mammalian Cells

(哺乳動物細胞における TRMT2A タンパク質の機能解明)

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Abstract

Background

Transfer RNA (tRNA) is the key adaptor molecule responsible for deciphering the three letters of the genetic code carried on mRNA, serving as the physical link between mRNA and protein during translation. A variety of nucleoside modifications occur during processing of tRNA, allowing the mature tRNA to function efficiently. More than 100 post-transcriptional modifications have been reported in tRNA to date. Many of these modifications are conserved across all domains of life, underscoring their biological importance. 5-methyluridine (m^5U or rT, ribothymidine) is one of the modifications in tRNA with the highest occurence. The ubiquitous presence of m^5U at position 54 in the TψC stem-loop of most eubacteria and eukaryotic elongator tRNAs implies a pivotal role of this modification. Intensive biochemical characterizations of m^5U54 has been performed in *Escherichia coli* and in *Saccharomyces cerevisiae*. TrmA and Trm2p are tRNA methyltransferases catalyzing m^5U54 in *E. coli* and *S. cerevisiae* respectively. Due to the lack of consistent phenotypic abnormalities, the biological function of these two proteins is still difficult to define. Not to mention the understanding to mammalian homologs remains elusive.

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Objective

I aimed to characterize the possible role and biological significance of tRNA methyltransferase 2 homolog A (TRMT2A), the mammalian homolog of TrmA and Trm2p by using TRMT2A overexpressing HeLa cell system and *Trmt2a* knockout mice model.

Material and methods

1) Establishing GFP-TRMT2A overexpressing HeLa stable cell lines to observe the effect of increased TRMT2A on cell growth properties and cell cycle progression.
2) Generating *Trmt2a* knockout mice to examine whether TRMT2A is essential in mammals and to clarify the pathological phenotypes in the absence *Trmt2a* gene.

Results

1) TRMT2A is the tRNA methyltransferase responsible for m^5U54 in mammalian cells

To clarify the role of TRMT2A on m^5U modification, total tRNA isolated from liver of *Trmt2a* knockout mice was subjected to HPLC analysis. The m^5U signal was completely lost in *Trmt2a* knockout mice, suggesting that TRMT2A is the enzyme responsible for m^5U modification in mammals.

2) TRMT2A has an inhibitory effect on cell growth and cell cycle

The cell proliferation of GFP-TRMT2A-HeLa cells was decreased compared to control cells. In addition, cell cycle profiles were altered, with an increased G2/M population. On the contrary, knockout of *Trmt2a* in MEFs led to increase of cell proliferation. These results imply that TRMT2A has an inhibitory effect on cell proliferation and cell cycle progression.

3) *Trmt2a* knockout mice showed a B cell defect

Trmt2a knockout mice are viable and fertile, exhibiting no overt phenotypes at the first glance. But the defects in B cells including the decrease of total B cell population, the altered B cell development in bone marrow, the affected B cell differentiation in spleen and the reduced immunoglobulin titers were observed in the steady state especially in aged mice, providing evidence for the essential role of TRMT2A in B biology.

Conclusion

These results shed light on the novel role of TRMT2A on cell proliferation, cell cycle profile and B cell biology, providing evidence that TRMT2A is a promising factors regulating mammalian cells in various cellular activities. Further systematic analysis of specific tRNA and genes altered by TRMT2A are required. Elucidating the role of TRMT2A and TRMT2A-catalyzed m^5U54 in translation levels will uncover the underlying signaling pathways of TRMT2A in both physiological and pathological conditions.