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論文の要旨
Abstract of thesis

Purpose

In this doctoral dissertation, Ms. Yu-Hsin Chang describes functional analysis of TRMT2A. The summary is as follows:

Transfer RNA (tRNA) is a key adaptor molecule responsible for deciphering the three-letter genetic code carrying on mRNA, serving as the physical link between mRNA and protein during translation. A variety of nucleoside modifications occur during the processing of tRNA, allowing the mature tRNA to function efficiently. More than 100 post-transcriptional modifications of tRNA have been reported to date. These tRNA modifications are well conserved across species, which highlights its biological importance. 5-Methyluridine (m5U or rT,ribothymidine) is one of the tRNA modifications at the highest incidence rate. The ubiquitous presence of m5U at position 54 in the TψC stem-loop of most eubacteria and eukaryotic elongator tRNAs implies a pivotal role in this modification. Biochemical characterizations of m5U54 has been intensively performed in *Escherichia coli* and in *Saccharomyces cerevisiae*. TrmA and Trm2p are tRNA methyltransferases catalyzing m5U54 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. The applicant, Ms. Yu-Hsin Chang,aimed to characterize possible role and biological significance of tRNA methyltransferase 2 homolog A (TRMT2A), the mammalian homolog of TrmA and Trm2p, by using aTRMT2A-overexpressing HeLa cell system and a TRMT2A-knockout-mouse model.

Methods

To observe the effect of increased TRMT2A on cell growth properties and cell cycle progression, the applicantestablished GFP-TRMT2A-overexpressing HeLa stable cell lines. In addition, TRMT2A knockout mice were produced to examine whether TRMT2A is essential in mammals and to clarify the pathological phenotypes of the knockout mice.

Results

1) TRMT2A is the tRNA methyltransferase responsible for m5U54 modification in mammalian cells
   To clarify the role of TRMT2A on m5U modification, total liver tRNA of TRMT2Aknockout mice was subjected to HPLC analysis. The m5U signal was completely lost in TRMT2Aknockout mice, suggesting that TRMT2A is the enzyme that catalyzes the m5U 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, the loss of TRMT2Ain MEFs led to the increase of cell proliferation. These results imply that TRMT2A has an inhibitory effect on cell proliferation and cell cycle progression.
3) TRMT2Aknockout mice showed a B cell defect
   TRMT2Aknockout mice were viable and fertile, exhibiting no overt phenotypes at first glance. However, 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 cell biology.

Discussion

The results obtained shed light on the novel role of TRMT2A regarding cell proliferation, cell cycle profile and B cell biology, suggesting that TRMT2A may be a promising factor 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 m5U54 at translational level uncovers the underlying signaling pathways of TRMT2A under both physiological and pathological conditions.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant proposesthe novel roles of TRMT2A in cell proliferation, cell cycle profile and B cell biology by using TRMT2A-overexpressing cells and TRMT2A-knockout mice. Although the applicant has found very interesting functions of TRMT2A, the molecular mechanism remains to be elucidated. Since TRMT2A appears to affect protein synthesis, and since B cells need to synthesize a large amount of immunoglobulins, it would be great if the applicant is able to demonstratethe change(s) of protein synthesis in TRMT2A knockout mice.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 29January,2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded a Doctor of Philosophy in Human Biology.