Cdk5rap1-mediated 2-methylthio-N^6-isopentenyladenosine modification is absent from nuclear-derived RNA species
(Cdk5rap1 媒介性 2-メチルチオ-N^6-イソペンテニルアデノシン修飾は核に由来する RNA 種には存在しない)

Background and Purpose: 2-Methylthio-N^6-isopentenyl modification of adenosine (ms^2i^6A) is an evolutionally conserved modification that is found in transfer RNAs (tRNAs). It has been recently shown that Cdk5 regulatory subunit-associated protein 1 (Cdk5rap1) specifically converts i^6A to ms^2i^6A at position A37 of four mitochondrial DNA-encoded tRNAs, and that the modification regulates efficient mitochondrial translation and energy metabolism in mammals. Curiously, a previous study reported that ms^2i^6A is present abundantly in nuclear-derived RNA species such as microRNAs, but not in tRNA fractions. To fully understand the molecular property of ms^2i^6A, the existence of non-canonical ms^2i^6A must be carefully validated.

Methods: Through mass-spectrometry, ms^2i^6A modification was determined in total RNA extracted from mitochondrial DNA deficient cells (ρ0 cells) of HeLa cells (human cervical cancer cells) and B82 cells (mouse fibroblasts) as well as HeLa cells and B82 cells. Monoclonal antibody recognizing ms^2i^6A was prepared and used to examine for the presence of ms^2i^6A in mitochondrial RNA and total RNA by Northern blotting. In addition, intracellular localization of ms^2i^6A was investigated by fluorescent immunostaining using ms^2i^6A antibody.

Results: In the present study, ms^2i^6A was analyzed in total RNA purified from human and murine ρ0 cells, in which mitochondrial DNA-derived tRNAs were completely depleted. The ms^2i^6A was not detected in these cells at all. A monoclonal antibody was generated against ms^2i^6A and ms^2i^6A was examined in murine RNAs using the antibody. The anti-ms^2i^6A antibody only reacted with the tRNA fractions and not with other RNA species. Furthermore, immunocytochemistry analysis using the antibody showed the predominant localization of ms^2i^6A in mitochondria and co-localization with the mitochondrial elongation factor, TUFM.

Conclusions: ms^2i^6A is not present in nuclear DNA-derived RNAs but exists only in four mitochondrial DNA-derived tRNAs (mt-tRNA). In addition, ms^2i^6A is present in mitochondria and co-localizes with mitochondrial protein translation machinery.