Lack of TRMT2A, a tRNA methyltransferase, leads to m^5U54 tRNA hypomodification and generation of tRNA-derived small RNAs

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Transfer RNAs (tRNAs) are subjected to a wide variety of post-transcriptional modifications to ensure their structural stability, correct folding, and efficient protein decoding. More specifically, modifications in the D- and T-loops of tRNAs are essential for tRNA stability. However, the biological role of the tRNA epitranscriptome in the generation of tRNA-derived small RNA fragments (tsRNAs), a class of small non-coding RNAs, is still not totally understood. The 5-methyluridine (m^5U) modification at position 54 of cytosolic tRNAs is one of the most common and conserved tRNA modifications among species. In mammals, this modification is catalyzed by the tRNA methyltransferase TRMT2A. To study the relevance of m^5U54 for tRNA-derived small RNA formation, we have knockdown TRMT2A in human cells and found that m^5U54 hypomodification resulted in ANG overexpression and tRNA cleavage near the anticodon, with accumulation of 5'tRNA-derived stress-induced RNAs (5'tiRNAs), in particular 5'tiRNA-Gly[GCC] and 5'tiRNA-Glu[CTC]. Moreover, we found that exposure to oxidative stress induces TRMT2A downregulation, ANG overexpression and tsRNA generation. Our results establish a direct link between tRNA demethylation and ANG-dependent tRFs formation and propose the m^5U54 as a tRNA cleavage protective mark.