Transfer RNA as a substrate of APOBEC3A deaminase

Karolina Hoffa-Sobiech

Abstract

APOBEC3A is a zinc-dependent cytidine deaminase whose main enzymatic activity involves catalyzing the deamination of cytidine to uridine in single-stranded DNA (ssDNA). In addition, APOBEC3A is capable of converting 5-methylcytidine and 5-hydroxymethylcytidine in ssDNA into thymidine and 5-hydroxymethyluridine, respectively. Under physiological conditions, APOBEC3A activity plays a key role in innate immune systems. Dysregulation of its activity may also contribute to tumorigenesis. For this reason, APOBEC3A has recently attracted particular attention from researchers. Its ability to target cytidine in RNA was discovered only a few years before the studies described in this work began. It involved the deamination of cytidines located within stem-loop structures in mRNA. This discovery opened the way for investigating the involvement of APOBEC3A in RNA editing. Considering that the stem-loop motif represents a key element of the secondary structure of tRNA molecules, and that APOBEC3A has evolved from tRNA deaminases, in this study was hypothesized that tRNA serves as a substrate for APOBEC3A. To verify this hypothesis, a method for producing APOBEC3A in a bacterial expression system was developed, and its activity on tRNA molecules was tested *in vitro*. Both quantitative analysis using ultra-high-performance liquid chromatography and qualitative analysis using next-generation sequencing demonstrated that, under *in vitro* conditions, APOBEC3A deaminated cytidines in human cell-derived tRNA molecules. Moreover, cytidine to uridine editing was detected in pools of tRNA fragments accumulated in cancer patients. In the subsequent stage of the study, it was shown that *in vitro* APOBEC3A deaminated 5-methylcytidine in a model mRNA molecule and in the anticodon loop of a synthetic tRNA-Leu-CAA. The results obtained significantly expand the current knowledge of APOBEC3A activity on RNA and provide a foundation for further research on the function of this enzyme in both physiological and pathological contexts.