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Review

MicroRNAs regulate APOBEC gene expression

Wei Cao[1] and Wei Wu[1,2]

[1]Translational Medical Center, Central Hospital Affiliated to Zhengzhou University, Zhengzhou, China and [2]Helen Diller Family Comprehensive Cancer Center, Department of Medicine, UCSF, USA

Summary. Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) is a family of evolutionarily conserved cytidine deaminases, encoded by eleven genes located in the human genome. APOBECs play key roles in innate immunity through their ability to mutagenize viral DNA and restrict viral replication. Recent cancer genomics revealed APOBEC3 subtype-mediated APOBEC-signature mutations are common in a broad spectrum of human cancers. The pervasive APOBEC3 activation in the host genome which converts cytosine to uracile during RNA editing has been suggested to depend on ATR/chk1 pathways. In this review, we highlight how microRNAs interact with the APOBEC gene family and post-transcriptionally regulate APOBEC gene expression, and we speculate how targeting specific microRNAs may reduce host genome mutagenesis via inactivation of APOBEC deaminases.

Key words: MicroRNAs, APOBEC, Mutagenesis, Mutation signature, DND1, P-bodies

Introduction

Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) is a family of evolutionarily conserved cytidine deaminases (Refsland and Harris, 2013). There are 11 human genes encoding members of the *APOBEC* protein family including: the activation-induced cytidine deaminase (*AICDA*) and *APOBEC1* on Chr 12p13.1, *APOBEC2* on Chr 6p21, seven *APOBEC3s* (*APOBEC3A, APOBEC3B, APOBEC3D/E, APOBEC3F, APOBEC3G, APOBEC3H*) on Chr 22q13.1, and *APOBEC4* on *Chr* 1q25.3 (Harris and Liddament, 2004). Except for APOBEC2 and APOBEC4, the other members of the APOBEC proteins normally function in (1) innate immune response to viral infection (e.g., HIV, HBV, HPV); (2) deamination of deoxycyditine (dC) to deoxyuridine (dU) in single strand DNA; (3) generation of somatic hypermutation in the process of cancer development. Recent studies support APOBEC3s activity is associated with tumorigenesis due to mutagenesis, in particular, APOBEC3B overexpressed in several human cancer types correlates with the presence of the APOBEC3B mutation signature (Burns et al., 2013; Roberts et al., 2013). Further study demonstrated that oncogenes, loss of tumour suppressor genes and drug-induced replication stresses activate transcription of APOBEC3B via an ATR/Chk1-dependent pathway in vitro. APOBEC3B activation can be attenuated through repression of oncogenic signaling pathways, small molecule inhibition of receptor tyrosine kinase signaling pathways and alleviation of replication stress through nucleotide supplementation (Kanu et al., 2016). However, the regulation of APOBEC gene expression is largely unknown. In this review, we will discuss how microRNAs may be involved in controlling APOBEC gene expression.

Offprint requests to: Wei Cao, Professor, M.D., Ph.D., Zhengzhou Central Hospital Affiliated to Zhengzhou University, 195 Tongbai Road, Zhengzhou 450007, P.R. China. e-mail: caoweiyu@hotmail.com

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The expression of APOBECs

The expression of AID/APOBECs is tissue and cell

type specific. AID was first discovered from a screen experiment between switch-induced and un-induced murine B lymphoma cells CH12F3-2 using a subtractive hybridization assay in 1999 and it is selectively expressed in activated B cells in germinal centers by immunization (Muramatsu et al., 1999). It is predominantly cytoplasmic. *Helicobacter pylori* infection in normal gastric epithelia can induce AID expression (Matsumoto et al., 2007). Overexpression of AID causes T cell lymphoma (Robbiani and Nussenzweig, 2013). APOBEC1, the first APOBEC family member to be identified and characterized, is expressed in the human small intestine and in the liver in rodents, this protein is involved in the editing of the apolipoprotein B pre-mRNA (Navaratnam et al., 1993; Teng et al., 1993). APOBEC1 presents in both nucleus and cytoplasm by virtue of an amino-terminal nuclear localization signal and a carboxy-terminal nuclear export signal. Overexpression of APOBEC1 causes hepatocellular carcinoma (Yamanaka et al., 1995). APOBEC2 expressed specifically in skeletal muscle and heart. Seven additional genes, or pseudogenes (designated APOBEC3A to 3G), were first identified as paralogs of APOBEC1 in 2002, and they are arrayed in tandem on chromosome 22 (Jarmuz et al., 2002). Jarmuz et al. (2002) also found that APOBEC3 gene expression is tissue-specific. In normal tissues, APOBEC3B expression was demonstrated in peripheral blood leukocytes. APOBEC3G was demonstrated in spleen, testes, ovary, and peripheral blood leukocytes. APOBEC3A showed hybridization to peripheral leukocyte RNA. APOBEC3C was demonstrated to express in spleen, testes, peripheral blood leukocytes, heart, thymus, prostate, and ovary at moderate to high levels and at low levels in several other tissues. No site of expression was found for APOBEC3D, APOBEC3E, and APOBEC3F. In tumor cells, APOBEC3B was abundantly expressed in colorectal adenocarcinoma and chronic myelogenous leukemia cells and to a lesser extent in melanoma, lung carcinoma, Burkitt's lymphoma Raji, and HeLa cells. This is consistent with recent evidence of overexpression APOBEC3B in multiple cancers including breast cancer (Burns et al., 2013; Roberts et al., 2013). APOBEC3G was expressed abundantly in colorectal adenocarcinoma and to a lower extent in Burkitt's lymphoma Raji cells, chronic myelogenous leukemia cells, and promyelocytic leukemia cells. APOBEC3C was expressed abundantly in colorectal adenocarcinoma and chronic myelogenous leukemia cells and to a lesser extent in melanoma, lung carcinoma, Burkitt's lymphoma Raji cells, lymphoblastic leukemia, HeLa cells, and promyelocytic leukemia cells. APOBEC3D expressed at modest levels in colorectal adenocarcinoma cells and chronic myelogenous leukemia cells, and at low levels in lung carcinoma and HeLa cells (Jarmuz et al., 2002). APOBEC4 is expressed in testis (Rogozin et al., 2005).

MicroRNA biology

MicroRNAs are 20-22-nt regulatory RNAs that participate in the regulation of various biological functions in numerous eukaryotic lineages, including plants, insects, vertebrate, and mammals. 1881 precursors and 2588 mature miRNAs have been identified in humans and mapped to GRCh38 (http://www.mirbase.org/cgi-bin/browse.pl?org=has). 60% of the genes in the human genome are predicted to be subject to miRNA regulation. The expression of many miRNAs is usually specific to a tissue or developmental stage, and the miRNA expression pattern is altered during the development of many diseases (Wu et al., 2007). Mature miRNAs are generated from RNA polymerase II-transcribed primary miRNAs that are processed sequentially by the nucleases Drosha and Dicer. Although miRNA can guide mRNA cleavage, the basic function of miRNA is to mediate inhibition of protein translation through miRNA-induced silencing complexes (miRISCs), all four of the Ago proteins (Ago1-4) are found to be present in miRISC. The guiding strand of miRNA in a miRISC interacts with a complementary sequence in the 3'-untranslated region (3'-UTR) of its target mRNA by partial sequence complementarities, resulting in translational inhibition (Sevignani et al., 2006). A 7-nucleotide "seed" sequence (at positions 2-8 from the 5'-end) in miRNAs seems to be essential for this action.

The miRNA-mediated translational repression consistently correlates with an accumulation of miRNA-bound mRNAs at cytoplasmic foci known as processing bodies (P-bodies). Several lines of evidence have indicated that P-bodies are actively involved in miRNA-mediated mRNA repression (Liu et al., 2005). The features of the P-bodies have: (1) the components of the P-body comprise of GW182 and Rck/p54 associated directly with Ago-1; (2) P-body formation is a dynamic process that requires continuous accumulation of repressed mRNAs; (3) P-bodies serve not only as sites for RNA degradation, but also for storage of repressed mRNAs. These mRNAs may later return to polysomes to synthesize new proteins.

APOBECs interact with microRNAs

There is little evidence to show how APOBECs genes are regulated. On the basis of some studies, it is reasonable to hypothesize that activation of APOBEC expression seems to be associated with viral infection or endogenous retrotransposons (Conticello, 2008). However, post-transcriptional regulation by microRNA machinery has been suggested from primate genome evolution, APOBECs and RNA interactions.

The genetic evidence indicated that a deletion polymorphism produces a hybrid, APOBEC3A_B, in which the APOBEC3A coding sequencing is fused to the APOBEC3B 3'-UTR. The deletion is rare in Africans

and Europeans (frequency of 0.9% and 6%), more common in East Asians and Amerindians (36.9% and 57.7%), and almost fixed in Oceanic populations (92.9%) (Kidd et al., 2007). The APOBEC3A_B carriers show more mutations of the putative APOBEC-dependent genome-wide signatures than cancers in non-carriers, conferring cancer susceptibility through increased activity of APOBEC-dependent mutational processes (Nik-Zainal et al., 2014). The APOBEC3A_B fusion product was expressed at much higher levels that a wild type of APOBEC3A product and had a potent hyper-editing activity on nuclear DNA (Caval et al., 2014). The possible biological interpretation is that APOBEC3A 3'UTR is targeted by one or more microRNAs that repress APOBEC3A expression, and that this repression is relieved in the APOBEC3A_B allele, in which APOBEC3A is instead fused to the APOBEC3B3'-UTR (Henderson and Fenton, 2015). To explore this hypothesis, we predicted potential microRNA binding sites in their 3' UTR using miRWalk 2.0 algorithm. The length of APOBEC3A 3'-UTR (NM_001270406) is 674 bp with 174 microRNAs (2 out of 4 prediction methods), while the length of APOBEC3B 3'-UTR (NM_001270411) is 356 bp with 82 microRNAs. The length of 3'UTR and microRNA binding sites may suggest APOBEC3A_B allele is an active form.

APOBEC3G is found in P-bodies and stress granules. It is associated with a high molecular mass structure (>700 kDa) in replicating cells, and this interaction is RNase-sensitive. Further studies indicate that APOBEC3G interacts with many RNA-binding proteins, among which are several miRNA-related proteins, such as Ago1, Ago2, Mov10, and poly(A)-binding protein 1 (PABP1). These interactions are either partially or completely resistant to RNase A digestion. Therefore, APOBEC3G function seems to be related to P-body-related RNA processing and metabolism. Recent developments have indicated that the function of P-body is closely related to miRNA activity, hence, APOBEC3G contacts the inhibition of protein synthesis by various microRNAs such as mir-10b, mir-16, mir-25, and let-7a (Huang et al., 2007). In addition, APOBEC3G is able to counteract RNA binding protein DEAD-END (DND1) repression and restore miRNA activity. APOBEC3G, by itself, does not affect the 3'-UTR of P27. APOBEC3G also blocks DND1 function to restore miR-372 and miR-206 inhibition through the 3'-UTRs of LATS2 and CX43, respectively (Ali et al., 2013). A more recent study showed APOBEC3G also interacts with lncRNA MALAT and microRNA2909 (Kaul et al., 2015). Taken together, microRNAs participate in APOBEC gene expression at post-transcriptional levels.

Conclusions

APOBEC enzymes, which have evolved as key players in natural and adaptive immunity, have been proposed to contribute to cancer development and clonal evolution of cancer by inducing collateral genomic damage due to their DNA deaminating activity. APOBEC gene expression is activated by viral infection and controlled by microRNAs in some scenarios. Some APOBEC members contact microRNA-mediated repression of translation by interact with Ago1, Ago2 and other proteins. It is speculated that manipulating specific microRNAs may control APOBEC gene expression, subsequently reduce the mutagenesis.

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Conflict of Interest. The authors declare no conflict of interest

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