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In cancer, A-to-I RNA editing can be the driver, the passenger, or the mechanic

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ABSTRACT

In recent years, A-to-I RNA modifications performed by the Adenosine Deaminase Acting on RNA (ADAR) protein family were found to be expressed at altered levels in multiple human malignancies. A-to-I RNA editing changes adenosine to inosine on double stranded RNA, thereby changing transcript sequence and structure. Although A-to-I RNA editing have the potential to change essential mRNA transcripts, affecting their corresponding protein structures, most of the human editing sites identified to date reside in non-coding repetitive transcripts such as *Alu* elements. Therefore, the impact of the hypo- or hyper-editing found in specific cancers remains unknown. Moreover, it is yet unclear whether or not changes in RNA editing and ADAR expression levels facilitate or even drive cancer progression or are just a byproduct of other affected pathways. In both cases, however, the levels of RNA editing and ADAR enzymes can possibly be used as specific biomarkers, as their levels change differently in specific malignancies. More significantly, recent studies suggest that ADAR enzymes can be used to reverse the oncogenic process, suggesting a potential for gene therapies. This review focuses on new findings that suggest that RNA editing by ADARs can affect cancer progression and even formation. We also discuss new possibilities of using ADAR enzymes and RNA editing as cancer biomarkers, indicators of chemotherapeutic drug sensitivity, and even to be themselves potential therapeutic tools.

Introduction to A-to-I RNA editing

A-to-I RNA editing was discovered 30 years ago and is one of the most studied transcriptome modifications (Bass and Weintraub, 1987; Rebagliati and Melton, 1987). However, only in the last few years has A-to-I RNA editing been possibly implicated in cancer formation and progression. Specifically, A-to-I RNA editing is the deamination of adenosine to inosine on double stranded RNAs (dsRNAs) (Fig. 1A), which is performed by ADAR (Adenosine Deaminase Acting on RNA) protein family (Bass, 2002). Inosine residues are recognized by the cellular machinery as guanosine. Therefore, A-to-I editing in coding regions can recode protein sequences and generate proteins with new functions (reviewed in (Nishikura, 2016)). Currently, there are three known ADAR proteins in mammals, ADAR1, ADAR2 and ADAR3. ADAR1 and ADAR2 are ubiquitously expressed, whereas ADAR3 is exclusively expressed in the brain (Melcher et al., 1996a; Melcher et al., 1996b). All ADAR proteins have two main domains, a catalytic deaminase domain at their C-terminal region and a number of dsRNA binding domains (dsRBD) at their N-terminal region ((Bass, 2002), (Thomas and Beal, 2017), Fig. 1B). The ADAR3 catalytic deaminase domain is probably non-functional (Melcher et al., 1996a). ADAR1 also has Z-DNA binding domains (ZDBD), whose functional activity is currently unknown (Herbert et al., 1997).

In addition, there are two more ADAR-related genes in mammals, ADAD1 and ADAD2, which are exclusively expressed in the testis and are enzymatically inactive with a yet unknown function (Schumacher et al., 1995). The ADAR1 gene encodes two main isoforms from alternative promoters, ADAR1p110 and ADAR1p150 (Fig. 1B). ADAR1p110 is the shorter isoform. It is constitutively expressed and mainly localized in the nucleus. In contrast, ADAR1p150 is mainly cytoplasmic and its expression is induced by the interferon pathway (Patterson and Samuel, 1995; Liddicoat et al., 2016). The ADAR2 gene is mostly expressed in the brain and is localized in the nucleus (Maas and Gommans, 2009). ADAR1 and ADAR2 were shown *in vitro* to homodimerize (Cho et al., 2003) and they differ in their editing targets. Specific editing sites that alter proteins were mainly found in the brain. One of the most studied edited transcript is that of glutamate ionotropic receptor AMPA type subunit 2 (GRIA2) (Sommer et al., 1991), which is part of a family of glutamate receptors that are sensitive to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and function as

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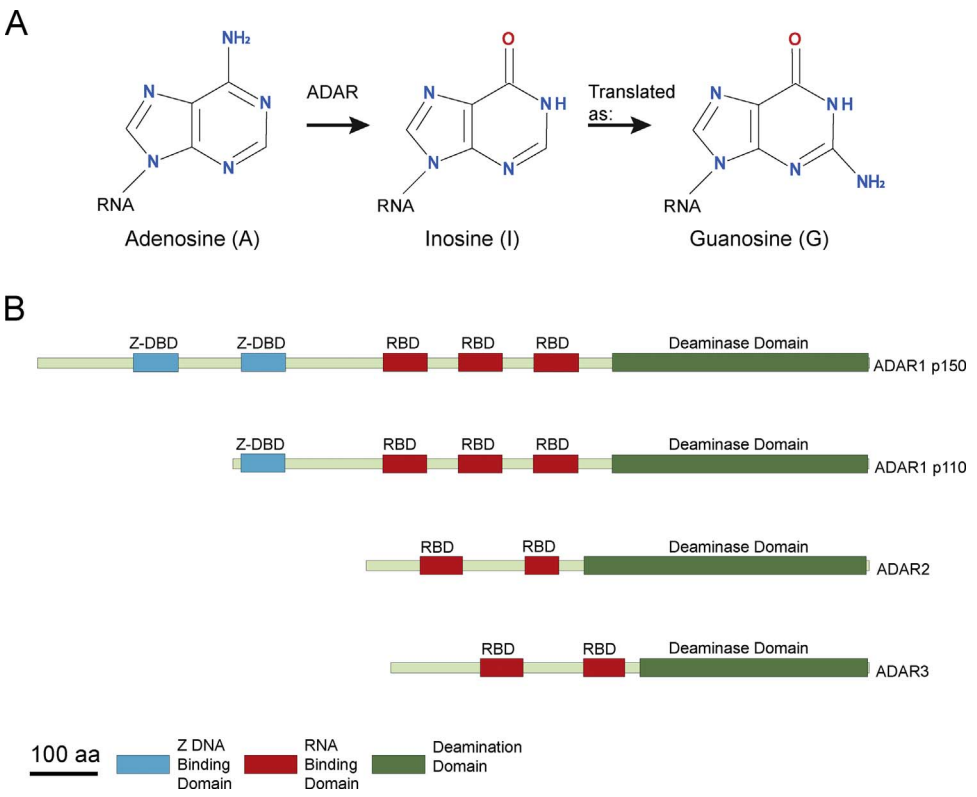

Fig. 1. Schematic representation of the deamination reaction and human ADAR protein family. (A) An amine group removal leads to the conversion of adenosine to inosine by an ADAR enzyme. Guanosine is shown to illustrate the structural similarity with inosine. (B) Members of the human ADAR family. The two main isoforms of ADAR1 are presented, ADAR1 p110 and ADAR1 p150. The deaminase domains are colored in green, the RNA binding domains (RBD) are colored in red and Z DNA binding domains (Z −DBD) are colored in blue. Proteins are scaled. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ligand-activated cation channels. These channels are assembled from 4 related subunits, GRIA1-4. RNA editing in GRIA2 is thought to render the channel impermeable to Ca^{+2}. Codon editing in GRIA2 subunit, which in some cases is edited to an extent of nearly 100% in transcripts, is needed to keep neuronal Ca^{2+} homeostasis. Indeed, mice harboring mutations in ADARs that lack the ability to edit these transcripts exhibit an early onset of epileptic seizures and premature death (Brusa et al., 1995). RNA editing can also alter splice sites and eliminate stop codons. Interestingly, ADAR2 edits its own pre-mRNA, resulting in a novel splice site that leads to a truncated protein due to the formation of a premature stop codon (Feng et al., 2006).

In recent years, advances in high-throughput sequencing led to the discovery that most editing events occur in clusters in non-coding repetitive transcripts, termed 'hyperediting', primarily in *Alu* repeat elements. In fact, editing events occur in more than half of the human transcriptome (Bazak et al., 2014). The level of editing (i.e., percentage of transcripts undergoing editing in a particular site) at site-specific coding sites is higher than that usually found in 'hyperediting' sites and is mostly associated with ADAR2 function.

Generation of microRNA can also be affected by A-to-I editing. Some pri-microRNAs undergo RNA editing and this editing prevents their processing and affects their ability to produce mature microRNAs (miRNAs) (Kawahara et al., 2007a; Yang et al., 2006). miRNAs can be edited at their seed regions, which can affect their binding efficiency to their targets and even alter their target specificity (Kawahara et al., 2007b). Editing in the target miRNA binding site can also affect the efficiency of the miRNA binding and regulate the target expression (Cho et al., 2017).

A-to-I RNA editing is essential in mice and humans. Mutations in ADAR genes as well as mutations that affect ADAR's editing functionality are associated with several human diseases. Mutations in the ADAR1 gene, mainly in its deaminase domain, are associated with the pathogenesis of dyschromatosis symmetrica hereditaria (DSH; OMIM # 127400). The latter is a rare autosomal dominant skin disorder characterized by hyperpigmented and hypopigmented macules on the face and dorsal aspects of the extremities that appear in infancy or early childhood (Zhang et al., 2004). ADAR1 mutations are also associated with Aicardi-Goutières syndrome (AGS; OMIM # 225750), an autosomal dominant autoimmune disorder characterized in its most severe form by cerebral atrophy, leukodystrophy, intracranial calcifications and chronic cerebrospinal fluid (CSF) lymphocytosis. This syndrome results from the chronic activation of type I interferon (Rice et al., 2012). Since editing that affects protein structure is mostly abundant in the nervous system, it is not surprising that changes in transcript editing levels were shown to result in neurological disorders including: Alzheimer's disease (Gaisler-Salomon et al., 2014), amyotrophic lateral sclerosis (ALS) (Hideyama et al., 2010), and autism spectrum diseases (Eran et al., 2013).

In the current review, we focus on the involvement of ADARs and A-to-I editing in cancer, including the ability to use editing sites as diagnostic biomarkers, and new developments of using ADAR proteins as druggable targets for cancer therapeutics.

The function of ADARs in development and immunity

Given the substantial amount of editing found in the human transcriptome, it is not surprising that RNA editing by ADARs has essential roles. Both ADAR1 and ADAR2 are required for normal development and knockout mice for either of these genes die early in development (Higuchi et al., 2000; Wang et al., 2000). The early embryonic lethality in ADAR2 knockout mice is partially due to the lack of editing in GRIA2 RNA, a glutamate ionotropic receptor (see above, (Higuchi et al., 2000)). ADAR1 knockout mice phenotypes led to our understanding of its possible important role in the innate immune response (Hartner et al., 2004; Wang et al., 2004). ADAR1 was found to be a key player in the suppression of the antiviral dsRNA sensing type I interferon pathway (Liddicoat et al., 2015) by preventing self-RNAs from triggering the innate immune response (George et al., 2016). ADARs can also be pro-viral for specific viruses (reviewed in (Samuel, 2012)). ADAR1 is also induced by the interferon response (Patterson and Samuel, 1995), which might be linked to the chronic inflammatory state observed in several types of cancer (Fumagalli et al., 2015). ADAR