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Review

Zalpha-domains: At the intersection between RNA editing and innate immunity

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ABSTRACT

The involvement of A to I RNA editing in antiviral responses was first indicated by the observation of genomic hyper-mutation for several RNA viruses in the course of persistent infections. However, in only a few cases an antiviral role was ever demonstrated and surprisingly, it turns out that ADARs – the RNA editing enzymes – may have a prominent pro-viral role through the modulation/down-regulation of the interferon response. A key role in this regulatory function of RNA editing is played by ADAR1, an interferon inducible RNA editing enzyme. A distinguishing feature of ADAR1, when compared with other ADARs, is the presence of a Z-DNA binding domain, Zalpha. Since the initial discovery of the specific and high affinity binding of Zalpha to CpG repeats in a left-handed helical conformation, other proteins, all related to the interferon response pathway, were shown to have similar domains throughout the vertebrate lineage. What is the biological function of this domain family remains unclear but a significant body of work provides pieces of a puzzle that points to an important role of Zalpha domains in the recognition of foreign nucleic acids in the cytoplasm by the innate immune system. Here we will provide an overview of our knowledge on ADAR1 function in interferon response with emphasis on Zalpha domains.

Contents

1. Introduction

In biology what you see is not always what you get. The discovery of RNA editing in Xenopus eggs in 1988 [1] and extensive research since have challenged a deeply rooted belief that the sequence of transcribed nuclear RNA is a faithful copy of the genomic sequence. This RNA modification was found to be present in all metazoans and was termed A to I RNA editing because it consists in the hydrolytic deamination of adenosines (A) toinosine (I) within RNAs. Inosine base-pairs with cytosine instead of thymine and so for any biological process that involves recognition of the RNA sequence through base pairing, inosine is an equivalent of guanosine. As a consequence, A to I editing is able to recode aminoacid sequences [2], to alter splice sites [3], create miRNA binding sites and change the specificity of miRNAs themselves [4].

Responsible enzymes for this RNA modification are adenosine deaminases called ADARs that show limited target sequence

Abbreviations: ADAR, adenosine deaminase acting on RNA; A to I, adenosine to inosine; PKR, protein kinase RNA depended; DAI, DNA depended Activator of IRFs; IFN, interferon; CNS, central nervous system; eIF2a, eukaryotic initiation factor 2a; HIV, human immunodeficiency virus.

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specificity [5]. Instead, their specificity is determined primarily by the secondary structure of the RNA substrate [6]. While ADARs modify a perfect RNA duplex in a promiscuous manner leading up to 50% of modified adenosines, the modification can be highly specific towards complex RNA structures containing bulges and internal loops [6].

The discovery that this RNA modification leads to functionally critical aminoacid changes in vertebrate neurotransmitter receptors like the GluR-2, GluR-5 and -6 subunits of AMPA receptors, the Serotonin-2C (reviewed in [2,7]) and the γ-amino butyric acid (GABA) receptors [8] as well as in invertebrate chloride channel subunits [9], nicotinic acetylcholine receptor (nAChR) subunits [10] and several other proteins involved in fast synaptic transmission [11], led to the notion that the primary role of A to I RNA editing is to provide the much needed diversity in the proteome of the animal central nervous system (CNS). This notion however left unexplained the observed abundance of RNA editing activity outside of the CNS.

Indeed, in recent years transcriptome analysis has shown that ADARs, the A to I RNA editing enzymes, are responsible for extensive modification of 3′UTR and intronic sequences of thousands of human genes [12–15] targeting secondary structures often created by pairs of inverted repetitive elements. These findings indicate that any extensive and stable pre-mRNA stem-loop structure is accessible to ADARs and a potential target for RNA editing and have led to a wide rethinking regarding the primary role of this post-transcriptional modification.

Double stranded RNA in the cytoplasm is known to represent a danger signal indicating viral infection and several antiviral pathways are triggered by dsRNA including the RNA interference pathway in invertebrates [16] and the interferon response in vertebrate cells [17]. Through the introduction of mismatches in RNA duplexes, A to I RNA editing can reduce their double stranded nature and mark such duplexes with Inosine. Could nuclear A to I RNA editing prevent the exposure of cellular mRNAs containing dsRNA structures to such antiviral pathways? Is RNA editing in the cytoplasm responsible for limiting the duration and/or the intensity of active antiviral responses? While evidence linking A to I RNA editing to antiviral responses dates back to its discovery, in the recent years several lines of research converge on an unexpected role for the editing machinery in the modulation of cellular responses to foreign nucleic acids.

2. Links between A to I RNA editing and immunity

The first evidence for a role of A to I RNA editing in immunity came with the discovery of modifications in several viral RNA genomes and viral transcripts. These modifications were shown to take the form either of genomic hyper-editing in the course of persistent viral infections or more specific RNA editing events in structured viral mRNAs. A second and independent line of evidence for a role of RNA editing in innate immunity comes from work implicating the interferon inducible ADAR1 editing enzyme in the regulation of the antiviral interferon response.

2.1. Modification of viral nucleic acids

Viral nucleic acids have been among the first known substrates of ADARs: the discovery of hyper-mutated RNA genomes of measles virus [18] and other members of the *paramyxoviridae* family was followed by similar observations for Vesicular Stomatitis Virus and mRNAs of the mouse polyoma DNA-virus reviewed in [19–21]. More recently similar hypermutation was observed in the invertebrate sigma RNA-virus [22] involving the ADAR enzyme of drosophilids. These findings were taken to suggest an antiviral role of ADAR mediated RNA editing, mechanistically based on scrambling the information content of viral nucleic acids, not unlike the role of the APOBEC3 family of cytidine deaminases in retroviral restriction. However, in only few cases an antiviral role of ADARs has been demonstrated and even in these cases it still remains unclear if this is a direct result of hyper-editing of viral nucleic acids or the result of the influence of ADARs on elements of the innate immune system. Indeed, knockdown of ADAR1 in an ADAR2$^{-/-}$ background in cell culture is shown to alter the cytopathic effects of polyoma virus infection [23] in a manner independent of the modification of viral RNA.

Specific editing within a well-defined sequence and structure context of viral nucleic acids was first demonstrated for Hepatitis D virus (HDV) [24]. Surprisingly, HDV was shown to make use of the cellular RNA editing machinery for its own needs: a stop codon in Hepatitis delta antigen is being changed to a tryptophan codon by RNA editing allowing the switch from the replication stage to the packaging stage, thus enabling, viral proliferation. Interestingly, HIV replication is also shown to be enhanced in cells over-expressing ADAR1, and HIV transcripts of Rev and Tat coding sequences as well as the TAR RNA are shown to be edited by ADAR1. However, it remains unclear whether the increased replication is editing depended [25–27]. Specific RNA editing has also been found in transcripts of the Kaposi sarcoma associated virus (KSHV) [28,29], where again RNA editing appears to be adapted by the virus as the levels of editing correlate with the replicative state of the virus [29].

In summary, for both the hyper-editing and the more specific editing events observed in viral nucleic acids there is no clear evidence that these base modifications overall represent an antiviral action of ADARs. On the contrary, for viruses like Hepatitis D, RNA editing represents a valuable cellular contribution.

2.2. ADAR1 as a regulator of antiviral responses

In vertebrate species we find two genes encoding for active dsRNA dependent adenosine deaminases: ADAR1 and ADAR2. ADAR1 was the first to be discovered and expression of an N-terminally extended form of the protein was shown to be upregulated during viral infection. Indeed, ADAR1 comes in two isoforms: a short, constitutively expressed and nuclear form (P110) and a longer, mainly cytoplasmic form (P150) which is transcribed from a different promoter responsive to type I and type II interferons [30] (Fig. 1A). The homozygous knockout of the ADAR1 gene in mice is embryonic lethal and its phenotypic characterization shows that its lethality is associated with extensive apoptosis in the hematopoietic tissue [31,32]. Further characterization of hematopoietic stem cells derived from an inducible ADAR1 gene knockout in mice shows a global upregulation of interferon responsive genes during embryonic development [33] pointing to a role of ADAR1 as a suppressor of interferon signaling. Selective knockout of the interferon controlled isoform P150 [34] suggests that this isoform is responsible for the observed embryonic lethality, although there is an ongoing debate on this matter.

The mechanism through which ADAR1 exerts control over innate immune responses has not been yet clarified. Clues however, have been obtained in studies showing that ADAR1 can antagonize protein kinase R (PKR) [35,36], a key protein of interferon response that mediates shutdown of cellular translation through the phosphorylation of eukaryotic initiation factor eIF2a during infection. PKR is activated by dsRNA and ADAR1 can inhibit its activation either through the sequestration of dsRNA or by rendering such dsRNA unrecognizable by PKR through modification. Indeed, ectopic ADAR1 expression results in a general increase in protein translation through the inhibition of PKR [37,38] and possibly of other dsRNA activated effector proteins of the interferon response