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RNA editing by Adenosine Deaminases Acting on RNA (ADAR) enzyme: Mechanism, Selectivity, and future prospect on RNA Therapeutics

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Abstract: Adenosine deaminases acting on RNA (ADARs) are a family of enzymes that catalyze the deamination reaction of Adenine (A) to Inosine (I) in double stranded RNA (dsRNA). ADAR1 and ADAR2 perform the catalytic conversion of Adenine (A) to Inosine (I) in humans. The X-ray crystal structure of the human ADAR2 deaminase domain bound to dsRNA reveals the flipping of the target adenosine (A) into the enzyme active site, which facilitates the ADAR editing. The structure also reveals how ADARs bind to the A-form double helical structure found in dsRNA. Classical Watson-crick dsDNA duplexes reside in the B-form double helix, with different helical pitch and phosphate backbone spacing, which explains why ADARs can't edit dsDNA. In contrast, the RNA/DNA hybrid adopts an A-form duplex that now allows ADARs to edit the DNA strand of RNA/DNA hybrids.

Introduction

The central dogma of molecular biology suggests the conversion of DNA to RNA, followed by RNA to protein for the flow of genetic information in a biological system. The process of the conversion of DNA to RNA is known as transcription whereas the synthesis of protein from RNA is known as translation. The Adenosine Deaminases Acting on RNA (ADAR) family of enzyme acts as a catalyst for the hydrolytic deamination of adenosine (A) to Inosine (I) in humans and other living organisms (Figure 1A).[1] The A to I deamination reaction by ADAR is substrate specific and recognizes only dsRNA for the deamination reaction.[1] In terms of Watson-Crick base pairing properties, Inosine behaves similarly as Guanine (G) and hence read as Guanine in cellular processes which includes splicing, translation and reverse transcription.[2] The mechanism (Figure 1B) of the deamination involves a step wise process. First a glutamate residue of the ADAR enzyme deprotonates the water bound with the Zn (II) ion present in the ADAR family of enzyme, which facilitates the nucleophilic attack on the C6 position of adenine followed by series of proton transfer results a high energy tetrahedral intermediate (Figure 1B). Liberation of ammonia from the tetrahedral intermediate converts A to I.[3] The ADARs are further subdivided into three categories as ADAR1, ADAR2 and ADAR3, among which ADAR1 and ADAR2 are catalytically active whereas ADAR3 is catalytically inactive.[1] There is no well-defined reason known for the catalytic inactivity of the ADAR3.[1] ADAR1 consists of two isoforms as ADAR1p110 and ADAR1p150.[1] The protein domain map of ADAR enzymes suggests the presence of a deaminase domain, dsRNA binding domain (dsRBD) in ADAR1 and ADAR2. The isoforms of ADAR1 consist of additional Z-DNA/RNA (Zα and Zβ) binding domain. The presence of a Nuclear Localization Signal (NLS) near to the third dsRNA binding domain (dsRBD3) of ADAR1 and at the N-terminus of ADAR2 facilitates the localization process of ADAR1 and ADAR2 in the nucleus.[1] ADAR1p110 consists of a Nuclear Export Signal (NES) near the Z-DNA binding domain which allows the movement of isoforms between the nucleus and cytoplasm.[4]

Effect of ADAR editing: The outcome of ADAR based RNA editing largely depends on the context, type of the RNA molecule to be edited and the tissues involved.[5] Conversion of A to I changes the codon and hence the amino acid substitution of the protein, which helps the formation of novel protein isoforms.[5] Several other non-coding events

like alteration of the secondary structure of RNA, RNA-protein interactions and stability of the RNA molecules are also affected by the ADAR editing of A to I.[6] Abnormal ADAR mediated A to I editing and the mutation in the ADAR gene is associated with several neurological disorders and cancer.[7] Different roles of ADAR in several types of cancer are leading towards the increasing interest of the development of ADAR based inhibitors as anticancer drugs.[8] Peter G. Zaphiropoulos et al. showed that output of the hedgehog signaling could be modulated using the RNA editing in GLI1 transcription factor.[8]

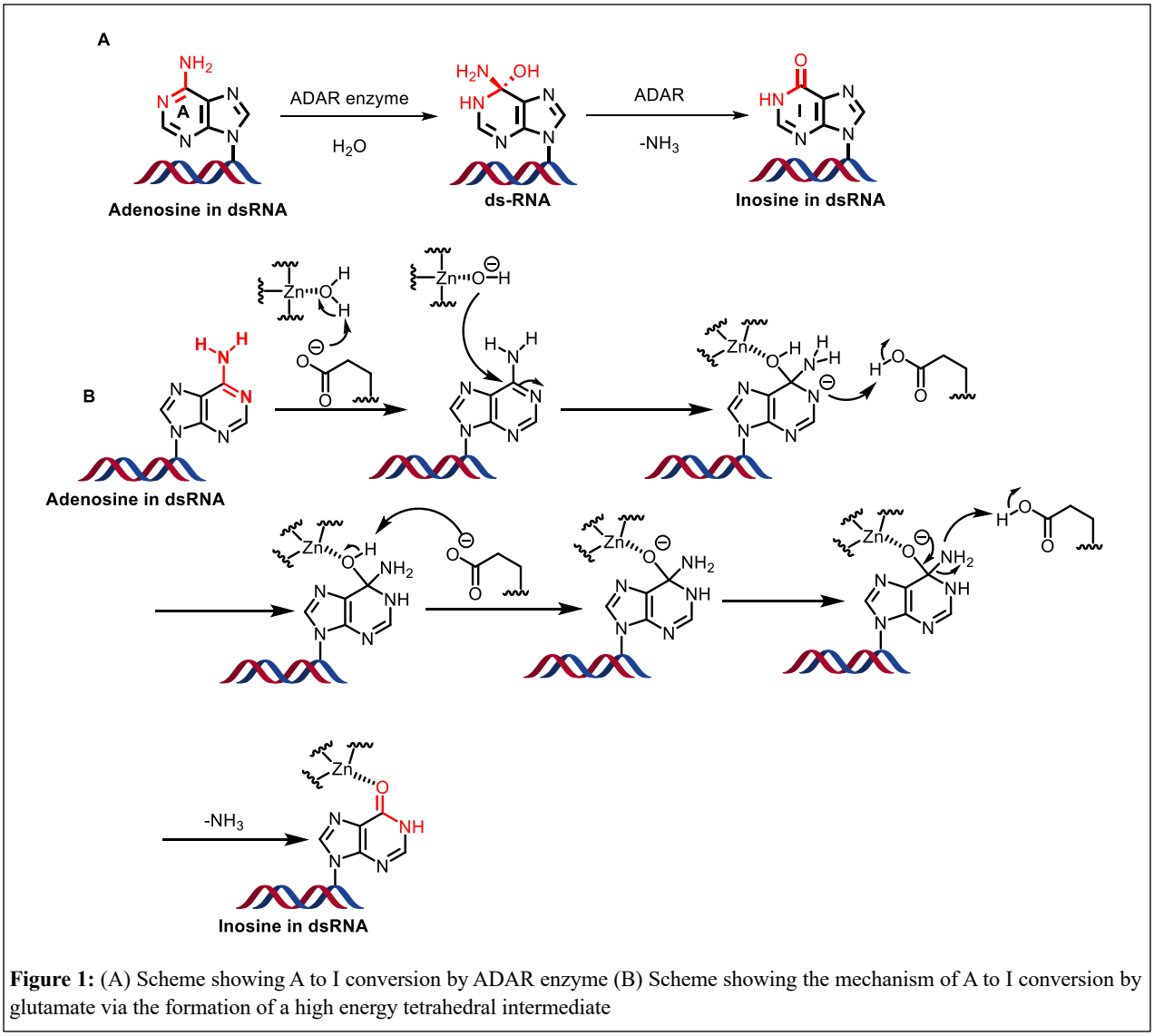

Figure 1: (A) Scheme showing A to I conversion by ADAR enzyme (B) Scheme showing the mechanism of A to I conversion by glutamate via the formation of a high energy tetrahedral intermediate

Site Directed RNA editing (SDRE): According to the Clinvar database (accessed on May 29, 2018) SP is responsible for 58% of genetic diseases.[9] Among which the major point mutation is G to A mutation (G is replaced by A), which causes the disease. The disease-causing G to A mutation could be reversed by ADAR based RNA editing.[10] An emerging therapeutic approach called Side Directed RNA Editing (SDRE) uses natural process of A to I editing by ADAR to correct the disease-causing mutation.[10] SDRE uses an antisense or guide oligonucleotide which is complementary to the target stand to form the duplex with the target. Recruitment of ADAR to the target editing site

and corrective A to I edit resulted the correct transcript which produces a functional protein. To achieve higher editing efficiency the current SDRE method uses chemically modified oligonucleotide (30-40 nucleotide long) with the endogenous human ADARs. Engineered ADAR proteins with improved efficiency and target specificity could also be used in SDRE.[10]

Crystallization of ADAR enzyme-dsRNA complexes: Useful information could be obtained by using modified nucleobases like 8-azanebularine (8-AzaN) as an ADAR target nucleobase instead of Adenine. 8-AzaN (Figure 2) is a mimic of Adenine (A) where the C8 carbon is replaced by nitrogen and the exocyclic amino group of Adenine (A) is replaced by hydrogen to inhibit the final ammonia liberation step.[11] Presence of electron withdrawing nitrogen atom at the C8 position and the replacement of the amino group of adenine (A) by hydrogen inhibits the liberation of the leaving group in the final step of the hydrolytic deamination of adenine and hence stabilizes the high energy tetrahedral intermediate.[11] Crystallization of the target strand modified with 8-aza nebularine (8-azaN) and the guide strand in presence of ADAR enzyme under suitable condition results crystal structure of ds-RNA bound with ADAR enzyme. Interestingly, the crystal structure reveals the mechanism of ADAR (ADAR2) based A to I editing. To be edited the target adenosine (A) flipped out of the ds-RNA to the active site of the enzyme, this is known as base flipping mechanism.[11] The flipped-out conformation of the ds-RNA is stabilized by a base flipping loop of ADAR2. The nucleobase opposite to the target adenosine (A) is known as orphan base. The amino acid residues of the flipping loop of ADAR2 intercalate into the space vacated by target adenosine and interact with the orphan base via hydrogen bonding interaction. Experimental data suggest a preference for ADAR editing across the Cytidine (C) and Uridine (U) orphan position due to a possible clash between the orphan base and the amino acid side chain.[11]

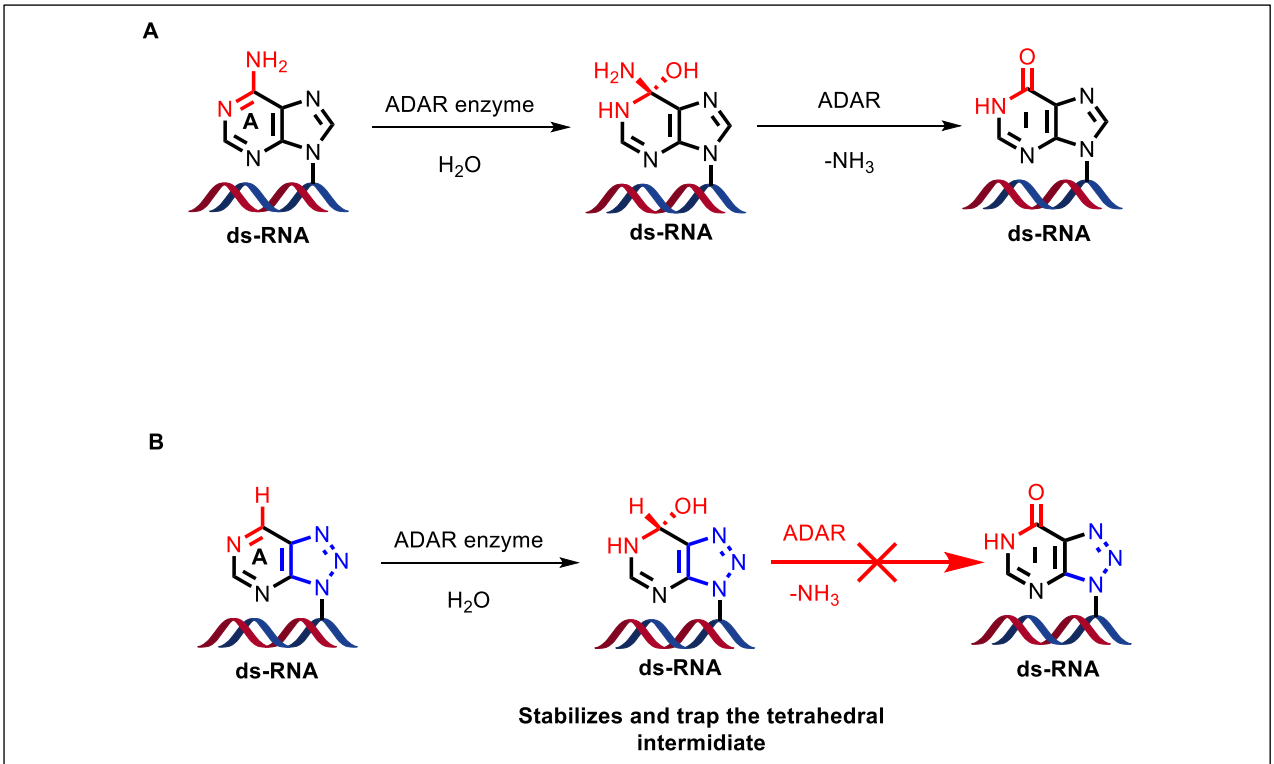

Figure 2: (A) Schematic representation of A to I editing involving Adenine (B) Schematic representation of the effect of introducing 8-azaN instead of target adenosine in ADAR mediated A to I editing.

Factors affecting ADAR editing: The nature of base present in the orphan position dictates the ADAR mediated A to I editing in ds-RNA. N3 nitrogen of cytosine (C) interacts with the protonated glutamate (E488) via intermolecular hydrogen bonding although the protonation and deprotonation of glutamate is pH dependent.[12] Mutation of the E488 protein to E488Q by replacing glutamate with glutamine results pH independent hydrogen bond interaction between N3 of cytidine (C) and the amide group of glutamine and enhances the deamination by enhancing the base flipping mechanism.[12] Establishing pH independent hydrogen bond interaction between the protein and the orphan base results higher rate of deamination reaction. Several other factors like the nature of the nucleic acid (LNA vs regular 2′-RNA modification like 2′-OMe, 2′-F etc.) at -1 position (Near to the orphan base) of the guide RNA gives interesting editing data and the editing of the target adenosine (A) could be suppressed by introducing a LNA modification at the -1 position of the Guide.[12] However detailed discussion of all the factors is out of the scope of this review.

Concluding remarks: Site Directed RNA editing (SDRE) by ADAR is an emerging therapeutic technique which could be used for repairing mutations and diseases at the RNA level. Interestingly It has been shown that ADAR edits the DNA strand of the preformed and hybridized DNA (*target*)-RNA (*guide*) hybrid duplexes (*A form of duplex*), hence ADAR mediated RNA editing could be used to edit the preformed DNA(*target*)-RNA(*guide*) hybrid duplexes.[13] With the scope of targeting RNA and DNA(*target*)-RNA(*guide*) hybrid duplexes ADAR is emerging as one of the important future therapeutic potentials in the field of oligonucleotide therapeutics.

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