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Neuberg, Dorotea

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The University of Rijeka, Department of Biotechnology,
Undergraduate programme "Biotechnology and drug research"

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Bachelor thesis

Dorotea Neuberg,
Mentor: Igor Jurak, Ph.D., Assoc. Prof.
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Sveučilište u Rijeci, Odjel za biotehnologiju,
Preddiplomski studij Biotehnologija i istraživanje lijekova

PROTEINI ADAR U BOLESTIMA LJUDI
Završni rad

Dorotea Neuberg,
Mentor: izv.prof.dr.sc. Igor Jurak
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In front of committee:

1. Elitza Markova Car, Ph.D., Associate professor
2. Ivana Munitić, MD. Ph.D., Associate professor
3. Igor Jurak, Ph.D., Associate professor

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Abstract

Adenosine deaminase acting on RNA (ADAR) is a mammalian protein family, consisting of five enzymes out of which ADAR1 and ADAR2 possess catalytic activity. Their mechanism of action, called A-to-I (adenosine-to-inosine) editing, is a post-transcriptional modification on double-stranded RNA (dsRNA) molecules and occurs in both nucleus and cytoplasm of various human cell. As one of the most abundant post-transcriptional modifications on nucleic acids, A-to-I editing is associated with various pathologic conditions throughout the human body. Because of their effect on dsRNA and subsequent pathogen recognition, ADAR proteins have a pivotal role in biochemical mechanisms of interferon (IFN) induction and regulation of the innate immune response. However, the central role of ADAR proteins in immunological mechanisms means that dysregulation of their activity, be it increase or decrease, can cause some significant disruptions in human body leading to autoimmune reaction or immunosuppression. Link between ADAR induced A-to-I editing and several cancer types is clearly present. However, in different tumors ADAR has different role. It can act as a tumor suppressor gene through neoantigen formation or stimulate tumor development as oncogene. Site-specific editing events in the central nervous system (CNS) are essential for the creation of glutamate and serotonin receptor subunits that make receptors impermeable to calcium. Editing in the CNS facilitates synaptic scaling and plasticity indicating that neural death by excitotoxicity is an evolutionary adaptation in case of ADAR2 failure. Moreover, dysregulation of ADAR activity is present in the neurodegenerative disease amyotrophic lateral sclerosis (ALS) and mental illnesses such as depression, bipolar disorder, schizophrenia and addiction. In a viral infection, depending on the cellular context and virus type, A-to-I editing can act both in a proviral and an antiviral manner. ADAR proteins can influence the course of viral infection in an editing-dependent or editing-independent form. In this review, we use the Epstein-Barr virus (EBV) and Hepatitis C virus (HCV) as exemplary cases to underline the essential role of ADAR in the viral life cycle.

Keywords:

adenosine deaminase acting on RNA (ADAR), A-to-I editing, cancer, central nervous system (CNS), excitotoxicity, proviral and antiviral effect

Sažetak

Adenozin deaminaza koja djeluje na RNA (ADAR) je porodica proteina sisavaca koja se sastoji od pet enzima od kojih ADAR1 i ADAR2 imaju sposobnost katalitičke aktivnosti. Mehanizam njihovog djelovanja, nazvan A-to-I editing, post-transkripcijska je modifikacija na dvolančanim molekulama RNA (dsRNA) koja se javlja se u jezgri i citoplazmi različitih ljudskih staničnih linija. Kao jedna od najzastupljenijih genetskih modifikacija, A-to-I editing povezan je s različitim patologijama u čitavom ljudskom tijelu. Zbog svog učinka na dsRNA i naknadnog prepoznavanja patogena, ADAR proteini imaju ključnu ulogu u biokemijskim mehanizmima indukcije interferona (IFN) i regulacije urođenog imunološkog odgovora. Međutim, središnja uloga ADAR proteina u imunološkim mehanizmima znači da poremećaj regulacije njihove aktivnosti, bilo to povećanje ili smanjenje, može uzrokovati značajne poremećaje u ljudskom tijelu, odnosno autoimune reakcije ili imunosupresije. Očita je veza između A-to-I editinga inducirano ADAR-om i nekoliko vrsta tumora. Međutim, u različitim tumorima ADAR ima različitu ulogu. Može djelovati tumor supresor gen i stvarati neoantigene ili poticati razvoj tumora kao onkogen. Editing događaji koji su specifični za mjesto središnjem živčanom sustavu (CNS) presudni su za stvaranje podjedinica glutamatnih i serotoninskih receptora koji čine cijele receptore nepropusne za kalcij. Uređivanje u CNS-u olakšava sinaptičko skaliranje, a plastičnost ukazuje da je neuronska smrt ekscitotoksičnošću evolucijska prilagodba u slučaju neuspjeha editinga proteinom ADAR2. Štoviše, poremećaj regulacije ADAR aktivnosti prisutan je kod neurodegenerativne bolesti amiotrofične lateralne skleroze (ALS) te mentalnih bolesti poput depresije, bipolarnog poremećaja, shizofrenije i ovisnosti. U virusnoj infekciji, ovisno o staničnom kontekstu i vrsti virusa, A-to-I editing može djelovati provirusno i antivirusno. ADAR proteini mogu utjecati na tijek virusne infekcije ovisno o uređivanju ili neovisno o uređivanju. U ovom pregledu koristimo Epstein-Baar virus (EBV) i hepatitisa C virus (HCV) kao ogledne slučajeve kako bismo razjasnili ulogu ADAR-a u životnom ciklusu virusa.

Ključne riječi:

adenozin deaminaza koja djeluje na RNA (ADAR), A-to-I editing, rak, središnji živčani sustav (CNS), ekscitotoksičnost, provirusni i antivirusni učinak

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Introduction to ADAR and A-to-I editing

A-to-I editing is one of the most common post transcriptional modifications in humans and it is being investigated with growing pace because of its evident connection with various diseases. Adenosine deaminases acting on RNA (ADARs) are a group of enzymes performing post-transcriptional epigenetic modification on double-stranded RNA (dsRNA). ADAR catalyses C6 deamination of adenosines (A) into inosines (I). Inosines (I) are decoded as guanosines (G) by multiple cellular proteins and structures, including ribosome and spliceosome because of preferential base pairing of inosine (I) with cytidine (C)¹⁻³. This mechanism, called A-to-I editing results in the change in function as well as in the three-dimensional structure of dsRNA because an I-U mismatch pair is less stable than an A:U base pair¹ and not complementary². So far, members of the ADAR protein family have been found in all metazoans³. One characteristics of A-to-I editing is its sequence-specificity. Both ADAR1 and ADAR2 have preference in targeting adenosines with 5' neighbor A or U and 3' neighbor U that is on the A/UAG site (the underlined base is the one being edited)³.

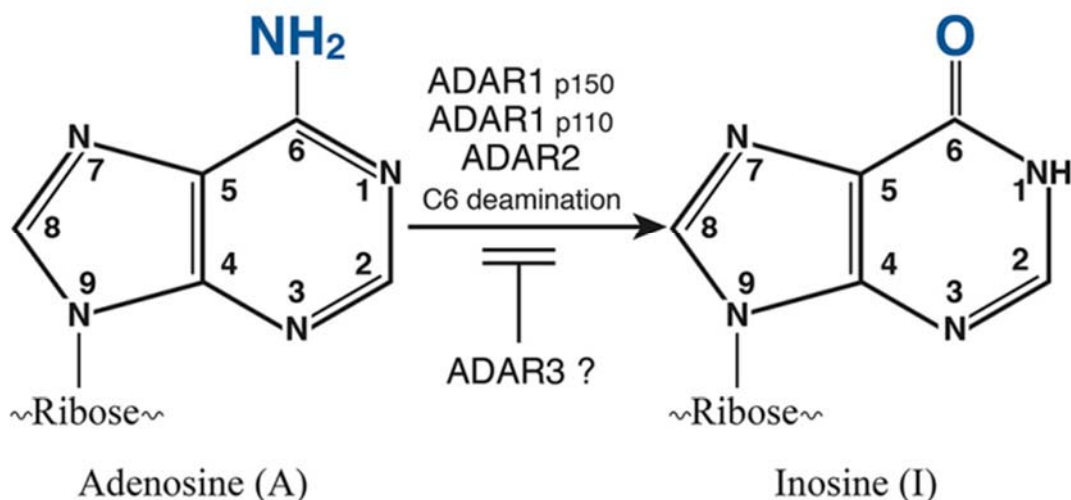


Figure 1: Mechanism of adenosine (A) deamination to inosine (I) adopted from Samuel et. al. ¹

Activity of this protein family is substrate-, cell-, tissue-, and time of development dependant. Two catalytically active isoforms of ADAR can be found in humans: ADAR1 (in truncated p110 variant and p150 variant) and ADAR2. Their action in the organism is quite different. Predominately, ADAR1 edits non-coding sites (for instance repetitive Alu sequences) while ADAR2 is responsible for editing coding sequences (such as the GluR2 subunit of glutamate receptors). Moreover, ADAR1 mostly performs hyperediting on extended dsRNA regions whereas ADAR2 edits site-specifically even though the exact principle of substrate selectivity is not yet known. Hyperediting is a term used for unspecific editing events happening in a grave extent on one sequence of dsRNA. On the other hand, site-specific editing means that editing events take place on a specific nucleotide(s) inside the sequence, generating specificity for one or more amino acid substitutions inside the coding sequence. ADAR1 p150 is the only isoform that is IFN-inducible and both nuclear and cytoplasmic, meaning that the p150 variant is able to edit dsRNA in the cytosol. Consequently, the p150 variant is capable of immune suppression via decrease of dsRNA, a well-known viral marker². The extent of editing itself varies widely, with some sites being site-specifically edited up to 99% in the human organism (GluR2 subunit of GRIA2) or just 1% hyperedited in other cases¹⁻³.

ADAR dysregulation has been recorded in various diseases affecting different organs and tissues of the human body. The major role of editing by ADAR1 is the suppression of innate immune response. Therefore, it is necessary to stress the dual role that dsRNA may have on the cell itself. It can act as a substrate for ADAR which in turn can suppress an immunological reaction, or as a trigger for innate immunity. A well-studied example of ADAR mutation is the Aicardi- Goutières Syndrome (AGS), an autoimmune child encephalopathy whose hallmark is an increased level of interferon (IFN). In case of AGS, an immunological reaction occurs because the editing of cellular (self) dsRNA is insufficient due to lack of ADAR with a functional deaminase activity³. Moreover, dysregulated ADAR1 and ADAR2

isoforms, can generate cancer-related neoantigens, with the addition of ADAR1 mediating cancer progression through suppression of cell immunity².

When stressing out the importance of ADAR proteins in disease pathology, it is important to mention a recent project that has integrated knowledge about editing events into the *Cell-line A-to-I RNA Editing Catalogue* (CLAIRE). CLAIRE is available at <http://srv00.recas.ba.infn.it/atlas/claire.html> and it provides a database of editing events in more than 1000 human cell line types. The aim of this online tool is to facilitate rational choice of appropriate cell lines in future research of editing⁴.

Purpose of the review

This thesis tackles the fine balance between ADAR proteins and immunological system in various human diseases. Viruses are included in the review as agents of various pathologies in human body. We will discuss biochemical mechanisms that share common characteristics in health – they are all antiviral and proapoptotic. On the other hand, intertwined with those same mechanisms, disruptions in ADAR proteins can be cause of pathogenesis.

1. ADAR proteins

There are five isoforms in the human ADAR gene family: ADAR1 and ADAR2, which have deaminase activity, ADAR3, without editing activity, ADAD1 and ADAD2, which are testis-specific and without catalytic functions. The ADAR homologues enzymes can also be found in invertebrates and they are functionally close to ADAR2 in vertebrates². ADAR1, ADAR2 and ADAR3 isoforms share similar domains, although their internal organisation varies significantly^{1,3}. They have a C-terminal deaminase domain responsible for the editing activity and a double-stranded RNA binding domain (dsRBDs), responsible for binding dsRNA³. The active ADAR is a dimer whose dsRBDs are homologous to dsRNA-binding domain in protein kinase R (PKR) and they can edit any double-stranded region which is at least 15-20bp in length². Interestingly, editing can occur also on a DNA strand in DNA-RNA hybrid³.

1.1. ADAR1

ADAR1 is both constitutively expressed and inducible by IFN. Both isoforms perform A-to-I editing and can be found in most cells. ADAR1 mostly edits non-repetitive Alu sequences of long dsRNA¹.

Gene-encoding *ADAR1* is placed on the human chromosome 1q21 and driven by multiple promoters out of which only one is IFN inducible while others are regularly active. Two size isoforms of ADAR1 are known so far: 1) ADAR1 p110 (approx. 110 kDa), that is constitutively expressed and localizes in the nucleus 2) ADAR1 p150 (approx. 150 kDa), inducible by IFN and localizes in the nucleus as well as in cytoplasm¹.

Catalytically active deaminase domain locates on the C-terminus of the protein. ADAR1 has three double-stranded RNA binding domains (dsRBDs), nuclear localization sequence and Z β domain. Difference between ADAR1 p150 and 110 isoform lies in Z α domain and nuclear export sequence (NES) on the N-terminus. Later enables p150 isoform to transport into cytoplasm (Figure 2)^{1,2}.

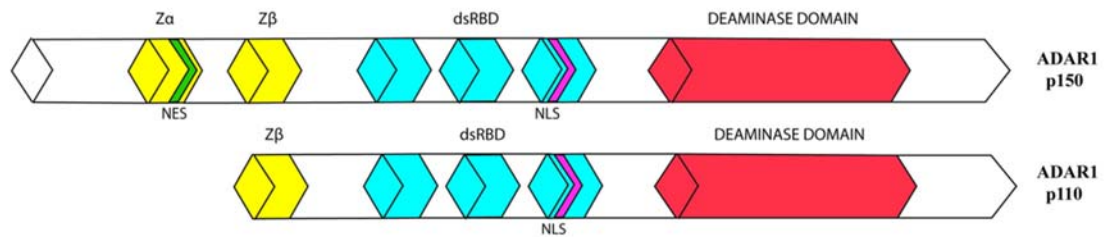


Figure 2. Organization of domains inside ADAR1 protein isoforms. Both isoforms of ADAR1 have a deaminase domain on the C-terminus (red), three dsRBDs (blue) and N-terminal Z DNA-binding domain – Z β (yellow) as well as a nuclear localization sequence (NLS) depicted in purple. The p150 isoform has a Z α N-terminal Z DNA-binding domain and a nuclear export sequence (NES) shown in green. (Adapted and modified from Gallo et al.³)

The role of Z DNA-binding domains in ADAR1 isoforms is not yet clear, however one suggested theory is that they guide ADAR1 to actively transcribed genes where Z DNA occurs frequently. The main reasoning for such an opinion is complementarity in shape and electrostatic -properties with Z-DNA. Z α domain binds Z DNA and Z dsRNA, which are thought to be natural targets of ADAR1. On the other hand, the Z β domain does not interact with Z DNA³. Furthermore, the Z α domain causes ADAR1 p150 to form a cytoplasmic stress granule even if the protein is not enzymatically active at that moment. The nuclear export signal (NES) enables ADAR1 p150 to localize in the cytoplasm, which is unique for the ADAR family. For that exact reason, truncated ADAR1 p110 is predominately nuclear with no ability of export¹.

1.2. ADAR2

ADAR2 localizes predominantly in the nucleus, although under specific conditions it can accumulate in the cytoplasm (reviewed in Gallo et al.)³. ADAR2 can be found in the central neural system (CNS) with specifically high expression in the brain. A-to-I editing events caused by ADAR2 are mostly site specific, highly selective and often regulated by neuronal excitation, and interestingly, linked to the circadian clock and sleep as well as neurodevelopment. Multiple size isoforms of ADAR2 can be found as a result of alternative promoter usage and alternative splicing¹.

Generally, ADAR2 has a simpler structure than ADAR1. As seen in figure 3 below, ADAR2 has two dsRBDs domains and a nuclear localization

sequence (NLS) responsible for its localization in the nucleus (Figure 3). All three above mentioned sequences contribute to the binding to dsRNA through direct interactions with 2'-hydroxyl group of RNA and through a small number of interactions with bases. Zinc (Zn^{2+}) cation can be found in the active site coordinated with histidine and two cysteines. Furthermore, a unique base-flipping mechanism of the deaminase domain was discovered recently^{5,3}.

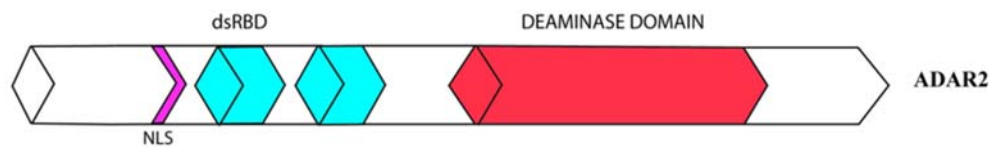


Figure 3: Organization of domains inside ADAR2 protein. ADAR2 has two double-stranded RNA binding domains (dsRBDs; blue) and nuclear localization sequence (NLS; purple). Deaminase domain with catalytic properties is on C-terminus (red). (Adopted and modified from Gallo et al. ³)

1.3. ADAR3

ADAR3 doesn't have deaminase activity and it doesn't catalyze A-to-I editing. It is limited to some regions of the brain such as the amygdala and the hypothalamus. There are hypotheses that ADAR3 serves as a modulator of ADAR1/2 activities through binding and direct isolation from shared targets¹. Evidence of this can be found in glioblastoma patients, where it inhibits editing through competition with ADAR2 for the binding site on GRIA transcripts⁶.

As seen in figure 4 below, except for the inactive deaminase domain, ADAR3 has two (dsRBDs) and a nuclear localization sequence (NLS). Additionally, ADAR3 is only isoform that has RG rich region at the N-terminus.

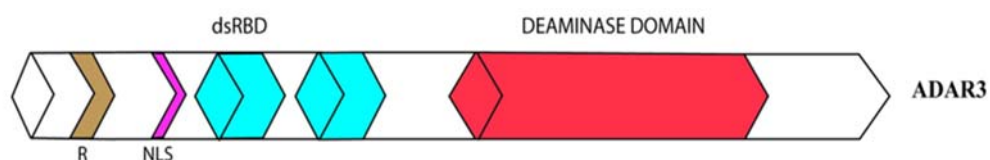


Figure 4: Organization of domains inside ADAR3 protein. ADAR3 has two double-stranded RNA binding domains (dsRBDs; blue), a nuclear localization sequence (purple) and a RG rich region at the N-terminus (brown). Deaminase domain without catalytic properties is on C-terminus (red). (Adopted and modified from Gallo et. al.³)

1.4. Roles od A-to-I editing

A-to-I editing, or processing of RNA transcripts possessing double-stranded structure, has an effect on multiple biochemical mechanisms, directly and indirectly causing changes in gene expression and protein structure. Some of the mechanisms that A-to-I editing has impact on are: mRNA translation, pre-mRNA splicing, RNA silencing, viral RNA replication and RNA structure¹.

A-to-I editing has a capacity to alter mRNA translation because inosine (I) that is produced in the deamination process of adenosine (A) is decoded as guanosine (G). The above described process can lead to amino-acid substitution in the proteins, out of which the best described are GluR2 and 5HT2cR subunits. Furthermore, this highly selective editing can lead to the elimination of the termination codon¹.

Another mechanism that A-to-I editing has a strong effect on is pre-mRNA splicing. Introns are mostly flanked by GT-AG splice site dinucleotides. Because I is being recognized as G, alternative splice sites can occur. One example of alternative pre-mRNA splicing in humans is ADAR2 transcript that is auto-edited in order to produce a functional protein trough splicing¹.

A-to-I editing can affect RNA silencing by microRNAs (miRNAs) and indirectly regulate gene expression on two levels: 1) processing of miRNA precursor; 2) editing of the seed sequence¹. miRNAs have the ability of gene expression modulation in two distinct modes: by inhibiting mRNA translation or by promoting mRNA degradation. Underlying the previous explanation, the impact of ADAR on this mechanism can affect the viral life cycle and genome itself as well as the human genome.

Viruses with an RNA genome have the potential to form dsRNA structures if complementary sequences are generated during replication while RNA-dependent RNA polymerase transcribes genes. In other words, the substrate for ADAR mediated A-to-I editing is formed. Viral genome mutation can then occur following novel (mutated) protein formation⁵,

increase viral sequence variety or leading towards a mutational catastrophe and loss of fitness .

Editing events change the dsRNA structure because an I-U mismatch pair is less stable than an A:U base pair. ADARs (namely p150 isoform), as a mechanism of immunotolerance edits the dsRNA, which is one of the triggers for pathogen detection, thereby suppressing interferon (IFN) induction and action, therefore suppressing an immunoreaction. Change in dsRNA structure caused by ADAR enables innate immune response suppression, no matter if the dsRNA comes from the host (self-dsRNA) or the pathogen (viral-dsRNA or non-self RNA)⁷. Figure 5 depicts various functional aspects of ADAR RNA editing.

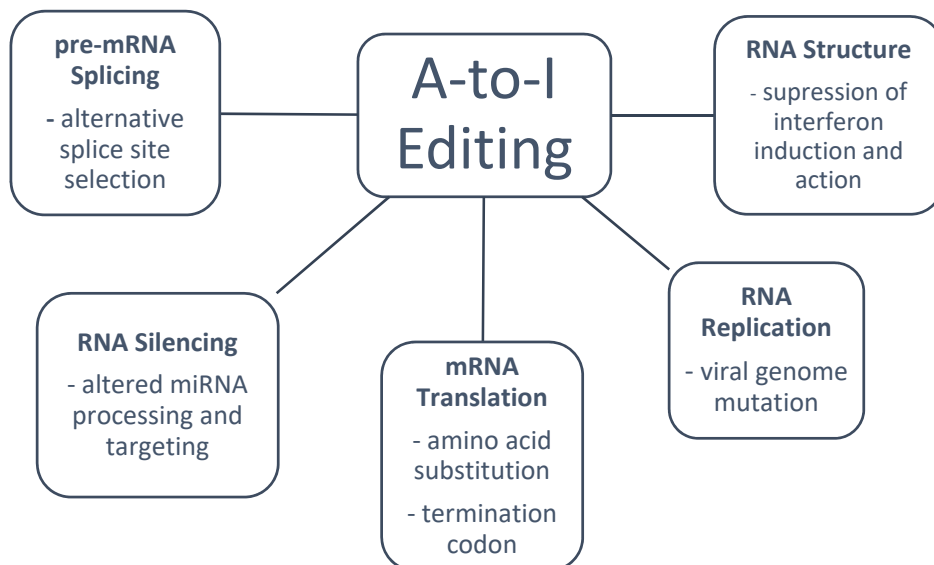


Figure 5: Schematic representation of biochemical mechanisms by which ADAR can affect gene expression and protein function. (adopted and modified from Samuel et al. ¹)

2. Biochemistry of ADAR proteins

In order to understand the role of ADAR proteins in human health and disease it is important to clarify the underlying cell mechanisms linked to ADAR proteins. This chapter will focus on biochemical mechanisms whose disruption in conjunction with ADAR may be crucial in understanding the pathogenesis of diseases. Most of these mechanisms use dsRNA as a sensor substrate for pathogen detection that starts a cascade of antiviral response.

2.1. Induction of ADAR1 p150 by IFN

The promotor for expression of the ADAR1 p150 isoform is inducible by IFN because of the consensus ISRE (interferon stimulated response element) sequence^{8,9}. The consensus JAK-STAT (Janus kinase – signal transducer and activator of transcription protein) signaling pathway is the core of ADAR1 p150 isoform induction. The first step is binding of IFN α or β to a cell-surface receptor which is found on most cells as one of the core components of innate immunity. Binding of IFN activates JAK1 and TYK2 (tyrosine kinase 2) kinases which mediate phosphorylation of STAT1 and STAT2 transcription factors. Transcription factors bind together and associate with the interferon regulatory factor 9 (IRF9). This complex is translocated to the nucleus, where it binds to the ISRE sequence and stimulates transcription of the p150 isoform.¹⁰ The ADAR p150 isoform edits cytoplasmic dsRNA and prevents activation of MDA5 – MAVS (melanoma differentiation-associated protein 5 – mitochondrial antiviral signaling protein), PKR (protein kinase R) and OAS – Rnase L signaling (Figure 6).

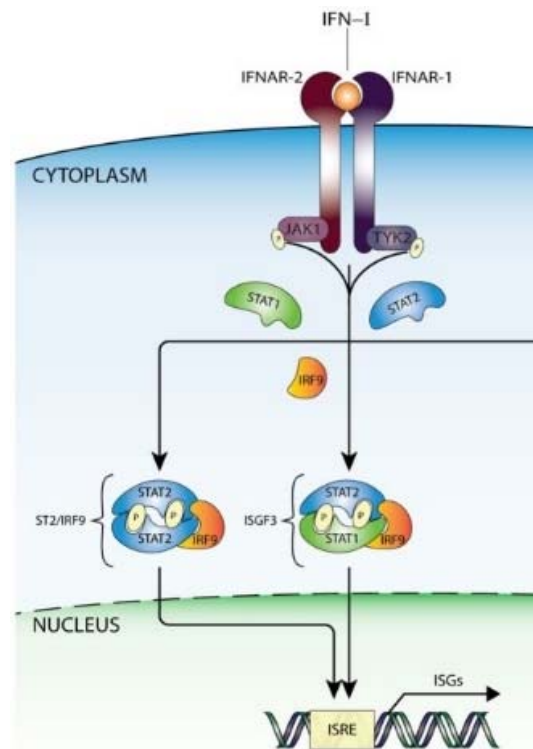


Figure 6: Activation of ADAR1 p150 isoform through JAK-STAT pathway. Interferon (IFN) binds to surface receptor and causes phosphorylation and activation of JAK1 and TYK2 kinases. Both kinases activate STAT1 and STAT2 transcription factors and cause their binding with IRF9 factor. Complex is translocated to nucleus where it binds to interferon-sensitive response element (ISRE) and causes transcription of IFN genes. (Adopted and modified from Michalska et al.¹¹)

2.2. MDA5 – MAVS

In normal conditions endogenous dsRNA is edited by ADAR proteins in the cytosol and not sensed by cytosolic sensors because of the structure destabilization. Cytosolic receptors for dsRNA are MDA-5 and RIG-I (retinoic-acid inducible gene I), subtypes of RIG-I-like helicase class of receptors. There is, however, a difference in the mechanism of dsRNA sensing between the two: MDA5 recognizes internal duplex structures while RIG-I recognizes the terminus of dsRNA. The role of both is to detect viral (nonself) dsRNA and induce the production of IFN¹². In the absence of ADAR1 cellular (self) dsRNA triggers MDA5-MAVS signaling because of a lack of editing activity. Activated MDA5 binds to the MAVS (mitochondrial antiviral signaling) adaptor protein. MAVS activates IRF-3 (Interferon regulatory factor 3), IRF-7 (Interferon regulatory factor 7) and NF-κB (nuclear factor kappa light chain enhancer of activated B cells) transcription factors and causes their translocation to the nucleus. NF-κB induces the

transcription of proinflammatory cytokines and IFN- α production, while IRF-3 and IRF-7 cause IFN- β production¹³. Signaling is terminated when the ADAR1 IFN inducible isoform p150 edits sufficient amount of dsRNA in the cytoplasm to stop signaling¹², that is, through a negative feedback mechanism.

2.3 Protein kinase R

Protein kinase regulated by RNA (PKR) is induced by IFN through a canonical JAK – STAT pathway. It is a serine/threonine kinase with two dsRBDs that shows antiviral and proapoptotic activities. The main proapoptotic mechanism is a general decrease in protein synthesis. Protein synthesis initiation factor eIF2 is one of the key drivers of the translation process. PKR is a sensor for dsRNA and binding of the later leads to PKR's dimerization and activation by autophosphorylation¹⁴. Activated PKR phosphorylates serine 51 on the α – subunit of eIF2 synthesis initiation factor which leads to suppression of the translation process. The main purpose of this cellular mechanism is to sense viral (nonself) dsRNA and prevent virus growth through disabling translational machinery. In normal cellular conditions, ADAR1 prevents hyperactivation of PKR through A-to-I editing of self-dsRNA. However, in the case of ADAR1 deficiency, PKR increasingly detects unedited self-dsRNA forms in the cytosol, meaning that the cell is going to enter apoptosis even though there is no viral infection¹⁴. Interestingly, ADAR1 and PKR share a conserved dsRNA binding motif¹⁵.

2.4. OAS – RNaseL

OAS-RNase L system is a proapoptotic and antiviral pathway inducible by IFN. There are three 2',5'-oligoadenylate (2-5A) synthetase enzymes (OAS) in humans: OAS1, OAS2 and OAS3. In multiple viral infections, the activation of RNase L is mainly OAS3 dependent¹⁶. OAS is a sensor for cytoplasmic dsRNA that synthesizes 2-5A from ATP when activated. RNase L activity depends on the dimerization after 2-5 oligo-A binding. Proapoptotic and antiviral properties of RNase L are based on endonuclease activity, that is, on viral and cellular RNA cleavage¹².

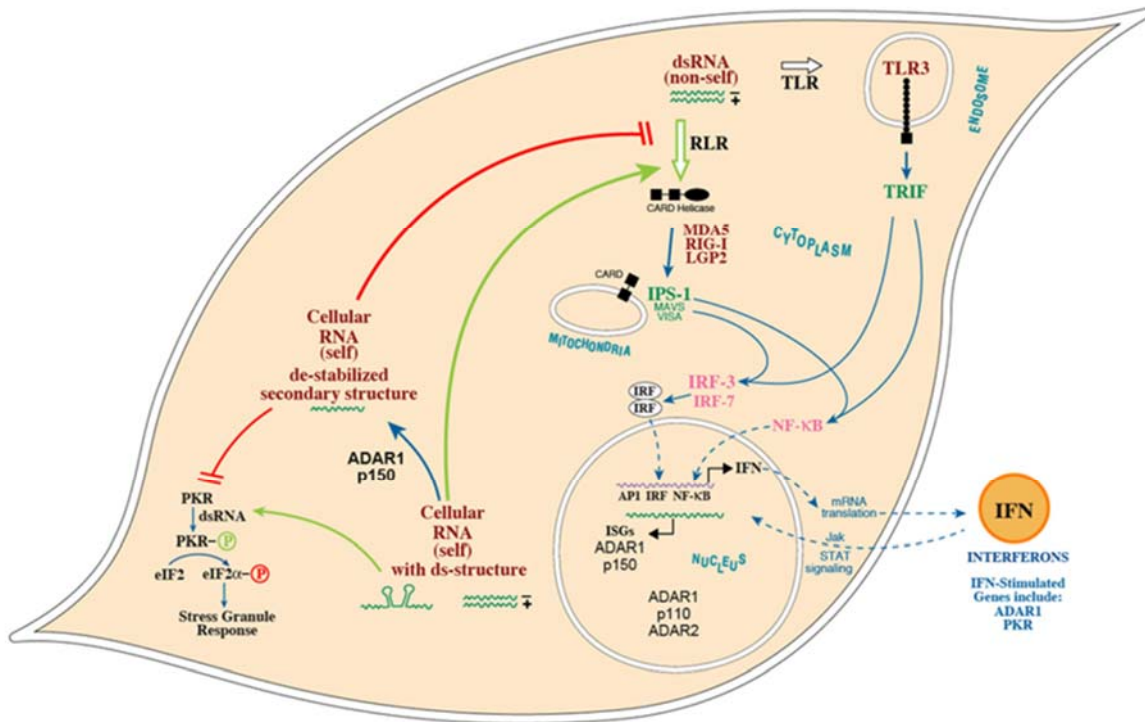


Figure 7: Cellular biochemical mechanisms linked to ADAR proteins. Unedited double-stranded RNA (dsRNA) activates MDA-5 and RIG-I which bind to mitochondrial antiviral signaling adaptor protein (MAVS). Complex activates NF-κB signaling pathway and IFN-α and IFN-β production. Protein kinase regulated by RNA (PKR) senses elevated levels of unedited non-self dsRNA in cytoplasm which lead to its autophosphorylation and activation. Activated PKR phosphorylates serine 51 on the α-subunit of eIF2 synthesis initiation factor which leads to suppression of the translation process. (Adapted from George et al.¹⁴)

3. ADARs in Cancer

A limited number of studies on ADAR proteins in cancer was published, reporting a number of effects which are opposing in different cancer types. Generally, there is no uniform consensus on ADAR's beneficial or adverse role in cancer. So far, dysregulation in ADAR activity has been noticed in several malignant diseases such as: breast cancer, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), metastatic melanoma, hepatocellular carcinoma, colorectal cancer and brain cancer³. Through this chapter we will discuss the central role of ADAR in cancer genesis and advancement¹⁷. Nevertheless, ADAR proteins can support formation of neoantigens harmful for tumors and slow down their progression². Therefore, ADARs can be perceived as both pro- and anti- cancer agents (Figure 8).

Author Herbert in review from 2019 describes cancer as a disease of both mutation and dysregulation¹⁷. To elaborate, mutations play a key role in the initiation and progression of disease, whereas dysregulation of physiological processes through epigenetic mechanisms ensures establishment of a favorable metabolic niche for cancer survival¹⁷. In summary, mutations on DNA are necessary to kick-start cancer development while alternations of cell mechanisms through epigenetic changes are essential for its maintenance and growth. It was not until recently that researchers began to focus on the latter, that is on the epigenetic mechanisms involved in cancer genesis. Editing affects varying fractions of the targeted transcript, leading to increased flexibility¹⁸. However, processes associated with cancer development are frequently contradictory² because of their need to establish a niche with blood supply, to spread and to evade both the innate and the adaptive immune system¹⁷.

Hundreds of cancer samples from multiple cancer tissues were characterized through transcriptome-wide analysis conducted by Paz-Jacov et al. Significant alternations of ADAR proteins and A-to-I editing were discovered in most cancer types. When compared to matching normal

tissues, in the majority of cancers editing was elevated. It is important to stress that in some cases editing is indeed downregulated. This study associates increased editing with patient survival and suggests classification of both DNA and RNA mutations as future diagnostics when determining patient's profile and treatment¹⁸. Similarly, Han et al. have systematically investigated landscape of A-to-I editing in 17 cancer types. A correlation with previously mentioned results is evident. A diversity of RNA-edited patterns in tumors relative to normal tissues was discovered, following ADAR alternations. Furthermore, noncoding regions that possess a number of clinically relevant editing sites were screened. Interestingly, RNA editing could be interlinked with drug sensitivity¹⁹. A few studies on the specific types of cancer will be described in more detailed below.

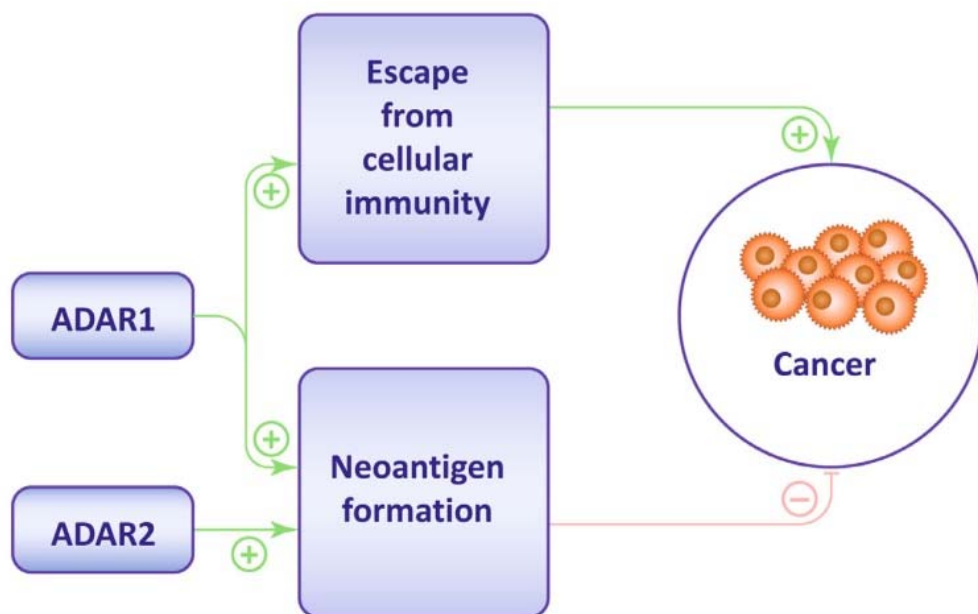


Figure 8: Dual role of ADAR proteins in cancer formation. ADAR1 can act as an oncogene through immunosuppression and as a tumor suppressor gene providing antigen formation. ADAR2 acts as a tumor suppressor in brain tumors through modulation of neural protein functions (adopted and modified from Goncharov et al. ²)

3.1. ADAR in breast cancer

Breast cancer impacts 2.1 million women annually worldwide, therefore being the most frequent type of cancer among women²⁰. ADAR1

induced A-to-I editing in breast cancer serves as an exemplary case of elevated non-specific editing that promotes tumor progression.

A-to-I editing is a principal resource of mRNA variability in breast cancer happening mainly in regions encoding Alu elements. Paired exome and transcriptome sequencing was conducted in 68 normal and cancerous breast tissues by Fumagalli et al. Consistent ADAR editing events were found on the same locus through normal and tumor tissues as well as in breast cancer cell lines. Furthermore, editing was increased in cancer cells when compared to normal controls. Correlation between ADAR1 elevation in samples and editing frequency was noticed²¹. Study by Paz-Yaacov et al. confirms this results, showing elevated levels of ADAR and A-to-I editing in cancer tissues when compared to normal tissues¹⁸. A higher level of ADAR1 in cancer samples was due to type I interferon response activation and ADAR DNA copy number amplification. Combined, they make 53% of expression variance. A silencing approach was applied and results show that reduction in ADAR leads to less proliferation as well as increased apoptosis. Importantly, ADAR1 wasn't associated with a specific breast cancer subtype. Editing sites in breast cancer therefore may represent new therapeutic targets for cancer treatment²¹.

3.2. ADAR1 in Chronic Myeloid Leukemia

Chronic Myeloid Leukemia (CML) is a cancer of the blood-forming tissue, or the bone marrow. In this particular case of leukemia, the bone marrow produces immature white blood cells called myoblasts in an extensive manner, therefore disabling development of erythrocytes and other blood cells²². A genetic background of CML is quite clear: rearrangement of genetic material fuses part of the ABL1 gene from chromosome 9 with BCR gene from chromosome 22. The end product is an abnormal fusion gene called BCR-ABL1 on the chromosome 22, now referred to as the Philadelphia chromosome (Ph chromosome²². CML has three phases: the chronic phase, the accelerated phase and the blast phase (crisis)²². In this review, a case of CML serves as an example of pro-cancer action of ADARs increased levels.

Whole transcriptome sequencing of normal, chronic phase and blast crisis cells from CML patients showed enhanced IFN- γ pathway gene expression together with BCR-ABL amplification. Additionally, enhanced expression of IFN-induced ADAR p150 isoform was detected during disease progression from chronic phase to blast crisis. Increased ADAR p150 levels were associated with upregulation of myeloid transcription factor PU.1 and downregulation of erythroid transcription factor GATA1, what confirms ADAR p150's involvement in the myeloid line changes. Knockdown of ADAR1 in CML cells led to a reduction of leukemia stem cells during serial transplantation. To conclude, ADAR shows overexpression on the CML and plays a central role in malignant progenitor self-renewal as well as in reprogramming of malignant progenitors to self-renewing leukemia stem-cells²³.

A conditional ADAR1 knockout mouse model (a model in which ADAR1 could subsequently be conditionally knocked-out) was used to determine whether Bcr-Abl transformed leukemic cells were ADAR1-dependent. To explain in further detail, the effect of ADAR deletion was monitored after leukemia establishment. Leukemia-initiating cells in CML can arise from a myeloid progenitor. ADAR1 knock-out caused rapid leukemic cell loss suggesting its pivotal role in leukemic cell survival. ADAR deletion has normalized peripheral white blood count and eliminated leukemic cells. Having in mind the aforementioned results, it is indicative that ADAR1 could be a new molecular target for CML therapy²⁴.

3.3. ADAR2 in Brain Cancers

The effect of ADAR2 editing in brain cancers, mainly glioblastoma, will be used as an example of tumor cell growth inhibition and anti-tumor action.

Generally, ADAR2 activity is impaired in glioblastoma (one form of astrocytosis). The GRIA2 Q/R-site has been reported as underedited in glioblastoma when compared to control tissues. Alterations in editing of 5HT2C receptor have also been noticed. Both previously mentioned alterations are in correlation with a decrease in ADAR2 enzymatic activity and suggest a role of A-to-I editing in tumor progression²⁵. Furthermore,

forced ADAR2 expression and subsequent editing was shown to prevent tumor growth in astrocytoma *in vivo* models²⁶. It has been reported that ADAR2 reduces expression of a large amount of miRNAs in glioblastoma, most of which act as oncogenes. Notably, editing of miR-222/221 and miR-21 precursors as well as a decrease in the expression of corresponding mature onco-miRNA *in vivo* and *in vitro* has significant effects on cell proliferation and migration²⁷. Evidence shows that the role of ADAR2 and A-to-I editing in glioblastoma is anti-tumoral on several levels.

4. ADAR editing in CNS

The ADAR2 enzyme site-specifically edits codons of the transcripts in the CNS. One of the earliest discovered substrates for ADAR in human genome were glutamate and serotonin 2C receptor transcripts¹. Consequently, substitution of amino acids can occur because inosine is decoded as guanosine by the ribosome machinery. This chapter focuses on the repercussion of multiple site-specific editing events in the CNS, including the mechanisms of specific diseases. As seen later, loss of function in human ADAR2 can lead to seizures, epilepsy, neurodegenerations, depression and mood disorders.

4.1. Link between ADAR2 and excitotoxicity, synaptic scaling and synaptic plasticity

Excitotoxicity is defined as neuronal dysfunction, degeneration and death caused by excessive glutamate level. Glutamate is an amino acid and the principal excitatory neurotransmitter in the mammalian CNS. It produces a majority of excitatory responses²⁸.

There are two types of glutamate receptors: ionotropic and metabotropic receptors²⁸. Three major ionotropic receptors activated by glutamate (iGluRs) are: N-methyl-D-aspartic acid (NMDA), α -amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainic acid (KA) receptors²⁸. Glutamate interacts with post-synaptic neuron receptors and gates ion channels permeable to various cations. The main importance of these receptors lies in mediation of fast glutamatergic synaptic transmission. The focus of the review are ionotropic receptors, AMPA and KA receptors specifically, because ADAR2 protein is in direct linkage with their malfunction and excitotoxicity development.

AMPA receptors (AMPA) consist of four subunits named GluR1-4 and require solely glutamate recognition for activation. The subunit composition governs specificity of cation influx which is variable. In contrast to others, subunit GluR2 removes calcium permeability. The AMPA receptor regulates depolarization of the membrane, what enables magnesium block removal from nearby NMDR receptors and consequently causes fast excitation²⁹.

Kainate acid receptors (KAR) consist of subunits named GluR5-7 (also referred as GluK1 and GluK2) and KA1-2. A flux of ions through them is allowed by glutamate application. Similar to AMPAR, they alleviate magnesium block from NMDR receptors²⁹. Co-assembly of above mentioned subunits, in both AMPAR and KAR, gives rise to a large number of receptor subtypes with distinct pharmacological and physiological properties³⁰.

Changing specific codons through dsRNA editing events has an important role on the CNS as a whole. One of the best described editing sites is the glutamine-to-arginine (Q/R) site in the GRIA2 transcript that encodes for the GluR2 subunit of AMPA receptor. In the GRIA2 transcript, glutamine codon (CAG) is present, but in mature mRNA an arginine codon (CGG) at the Arg607 site can be found. This same pattern is present in GluR5 and GluR6 subunits of the KA receptor, making this editing event present in two glutamate receptor classes. Difference between classes can be seen in the scope of editing - GluR2 is edited with 99% efficiency while GluR5 and GluR6 subunits occur in both edited and unedited versions³¹. Ion channels with edited GluR2(R) subunit are constituents of natural AMPA receptors and impermeable to calcium³¹. Lack of editing events at Arg607 leads to the creation of GluR2(Q) subunit, that is, constituent part of AMPA receptor, permeable to calcium³². The importance of editing at the Q/R site confirms a study conducted on the mice pups homozygous for functional null allele (ADAR^{-/-}) for Adar2 enzyme. This mutation is lethal for mice pups - they die young and with seizures. However, mutant mice death is prevented when Adar2 loss is combined with mutation of GluR2(R), that is, with already edited version of GluR2 subunit of AMPA receptor³³. To summarize findings, site-specific editing of AMPA receptor's GluR2 subunit has crucial role in viability and calcium flux in neurons.

Evidence shows that site-specific editing in subunits of ionotropic glutamate receptors regulates their trafficking to the cell membrane as well as their function. In CNS, trafficking is a central mechanism that underlies activity dependent forms of synaptic plasticity³⁴. Furthermore, trafficking is a mechanism through which modulation of synaptic strength (synaptic

scaling) is achieved³⁵. AMPARs are the main fast transduction elements also critical in the expression of plasticity³⁶. Aforementioned GluR2(R) subunit of AMPAR is retained and stably resides inside the endoplasmic reticulum (ER) due to the Q/R-editing site in the channel-lining pore. If this site remains unedited, GluR2(Q), which is permeable to calcium, efficiently transports to the cell membrane surface³⁴, meaning that the calcium flux during depolarization of the neuron will be more powerful. Moreover, editing affects the assembly of receptor tetramers³⁵. KAR upscaling (increase in the number of receptors in order to counteract loss of synaptic activity³⁷) and trafficking is dependent on neural activity³⁸ and ADAR2³⁷. Suppression of synaptic activity is followed by KAR upscaling which is a consequence of proteasomal ADAR2 degradation that causes GluK2 editing reduction. GluK2(Q) subunits exit the ER more efficiently, causing KAR upscaling on the surface³⁷. It is important to note that this mechanism is specific to KARs and it doesn't have a role in the editing status of GluR2³⁷. Editing of GluK2 does not affect trafficking directly³⁹ as in case of GluR2.

It is indicative that neurons have evolved to increase the risk of neural death through excitotoxicity if ADAR2 editing in cells fails. Both in the case of KAR and AMPAR Q/R site-edited subunits accumulate in the ER suggesting that ADAR2 editing evolved to keep back glutamate signaling by synaptic scaling in response to general neural excitation. Buckingham et al. call GluR2 (GRIA2) subunit a "gatekeeper" for motor neuron survival because it can literally switch between two cellular phenotypes distinct in their vulnerability to excitotoxicity³⁰.

4.2. ADAR2 in neurodegeneration – Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by a selective loss of upper and lower motor neurons⁴⁰. Neuron death by glutamate excitotoxicity is considered to be its cause. There are several molecular causes in the background of ALS such as superoxide dismutase 1 (SOD1) and optineurin mutation.

Lack of GluR2 mRNA Q/R site editing is also a molecular hallmark of ALS. Editing varies deeply among neurons of each individual with ALS (from 0% to 100%) and it is selective to ALS critical regions of the CNS⁴⁰. The cause of this downregulation is absence or lack of ADAR2 protein. Studies on the knock-out ADAR2 mouse strain that shared symptoms of ALS showed that all motor neuron death can be prevented by substituting wild-type unedited GluR2 alleles for alleles point-mutated to express Q/R site edited GluR2 in the absence of ADAR2⁴¹. Expression of ADAR2 is significantly downregulated in all motor neurons of ALS patients, especially in those expressing unedited GluR2 mRNA, meaning that ADAR2 downregulation is a pathological change relevant to ALS pathogenesis⁴². Downregulation of ADAR2 protein in ALS induces mislocalisation of TAR DNA-binding protein (TDP-43) through Ca²⁺-dependent serine protease. TDP-43 N-terminus aggregation can be seen as another molecular hallmark of ALS which is concomitant to ADAR2⁴³. Furthermore, autophagy flux was increased in degenerating ALS motor neurons, mostly from Ca²⁺ overload due to lack of ADAR2 editing, as seen on the ADAR knockout mice study. On the other hand, mice genetically modified to express edited GluA2 did not show increased autophagy flux⁴⁴. This evidence shows that ADAR2 downregulation or deficiency are indeed interlinked with molecular mechanisms in the background of ALS.

4.3. Mental Illnesses and Addiction

5-HT_{2C} serotonin receptor (5-HT_{2CR}) is the only G-protein-coupled receptor so far known to undergo RNA editing and considered to be involved in pathophysiology of several mental illnesses such as schizophrenia, major depressive disorder and anxiety. There is a total of five editing sites in exon V, a specific region that is responsible for encoding a second intracellular loop of the receptor, crucial for G protein coupling⁴⁵. Both ADAR1 (on adenosine sites A, B, C and E) and ADAR2 (on adenosine site D and E) act in editing events. Editing permutations lead to 24 variants of 5-HT_{2CR} with different activity. Edited forms of 5-HT_{2CR} seem to have decreased activity, likely because of low capacity to couple G-proteins⁴⁵. Furthermore,

it seems that editing has an inhibitory effect on downstream G-protein-mediated pathways proportional to the extent to editing⁴⁵.

Research so far is not consistent: while some report disease-specific alternations in exon V, others report no correlation. It has been shown that A-to-I editing of 5-HT₂CR is altered in cortical areas involved in mood regulation and decision making in depression-related suicides when compared to healthy individuals. These findings suggest that region-specific changes in RNA editing of 5-HT₂CR mRNA contribute to the etiology of major depressive disorder and suicide⁴⁶. VGV mice, that express the exon V fully edited, show anxiety and depression-like behaviour⁴⁷. A direct link between A-to-I RNA modifications of peripheral markers and A-to-I editing-related modifications in the brain has been discovered. This could shed a new light on the identification of a blood-based markers for suicidal behavior⁴⁸ and treatment-emergent depression in HCV patients⁴⁹.

As discovered in postmortem samples, ADAR2 expression tends to be decreased in brains of patients with schizophrenia and bipolar disorder⁵⁰. In schizophrenia, sites in genes encoding AMPAR and postsynaptic density proteins were less edited⁵¹. On the other hand, genes encoding translation initiation machinery were edited more in individuals with schizophrenia, mostly sharing common sequence motifs and overlapping with binding sites for RNA-binding proteins crucial for neurodevelopment⁵¹. Association of around one third of editing sites with cis-regulatory variants has been found⁵¹. Moreover, altered editing of AMPA glutamate receptors, caused by ADAR2 down regulation, seems to play a role in the pathophysiology of mental illnesses, schizophrenia in particular⁵⁰.

Various evidence shows connections between editing in brain and addiction behavior, although they come from rodent models. Investigation on mice models has shown link between chronic ethanol consumption and increased level of editing of 5-HT₂CR in nucleus accumbens (NAc). NAc-specific ADAR2 knockout mice model shows that editing in the NAc is involved in the creation of alcohol preference after chronic alcohol consumption⁵². Furthermore, increased ADAR2 expression in the shell of

NAc increased GluA2 Q/R site editing and surface expression of GluA2 containing AMPAR which promoted cocaine seeking⁵³.

5. Role of ADAR in viral infection

All viruses that have dsRNA structures at any stages of their life cycle may potentially undergo RNA editing events mediated by ADAR enzymes. Up to now, A-to-I editing has been discovered in multiple viruses including: Murine polyoma virus, Epstein-Barr virus (EBV or HHV-4), Hepatitis C virus (HCV), Dengue virus, Human immunodeficiency virus type 1, Hepatitis delta virus, Influenza A virus, Measles virus, Vesicular stomatitis virus and many more. Modifications can appear as hyperediting or site-specific editing. Furthermore, ADARs can affect viral genome in an editing dependent or independent manner⁵⁴.

Evidence from various research shows that the effect of ADARs on the virus-host interaction can be proviral or antiviral. Decisive factors are the specific animal virus and mammalian host cell combination and the type of ADAR protein expression⁵. In cases of some viruses, ADAR can act both in a proviral and antiviral mode, depending on the molecular pathways activated in the host cell⁵⁴. The best candidate for editing of viral substrates is definitely ADAR1 p150 because it localizes in the nucleus and cytoplasm⁵⁴.

5.1. Editing-dependent and -independent effects

There are two modes in which ADAR proteins can influence course of viral infection: the editing-dependent and -independent manner. ADAR's editing activity can directly affect viral RNA in a way that impacts the outcome of viral infection, or it can indirectly affect cellular RNA in manner that alters cellular production that subsequently impacts viral-host interaction (more indirect)⁵. Furthermore, ADAR proteins can impact the outcome of viral infection in an editing independent way, that is, by altering protein or nucleic acid binding interaction⁵. ADAR as a protein structure physically prevents the proper functioning of nucleic acids or other proteins. So in this case, the impact on the viral infection is due to the intermolecular interaction, and not to the editing of a nucleic acid of some kind.

5.2. Antiviral role

The conclusion that ADAR proteins can have an antiviral role is based on the few pieces of evidence from previous findings, all having in common a link to the host defense mechanism against viral infection. Firstly, most viruses replicate in the cytoplasm and the ADAR p150 (the only isoform that localizes in the cytoplasm) is likely the best candidate for editing most viral dsDNA⁵⁴. Finding made by Patterson and Samuel in 1995 that ADAR p150 expression can be induced by IFN additionally supports this hypothesis⁵⁵. Secondly, ADAR p150 physically interacts with the nuclear factor 90 (NF90) proteins which consequently activate cellular antiviral cascade and induce IFN- β production⁵⁴. Thirdly, several viral RNA genomes and transcripts show A-to-I hyperediting during lasting infection leading to frequent mutations in viral RNAs⁵. We can conclude that there is a line of evidence supporting ADAR's role in the host defense mechanism.

5.3. Proviral role

In contrast to the previously mentioned antiviral role, some lines of evidence show that ADAR enzymes can act as proviral factors with special emphasis on ADAR1 as an enhancer of viral infection⁵⁴. Two mechanisms are held accountable for ADAR's proviral role: 1) editing viral dsRNA substrates¹⁵ and 2) inhibiting double-stranded RNA-dependent protein kinase (PKR) in an editing-independent manner⁵⁶. The above-mentioned mechanisms can act independently or in synergy.

There are several mechanisms by which viruses can inhibit PKR antiviral activity described by Gale and Katze: 1) suppress PKR activity by direct binding, 2) induce PKR degradation, 3) promote eIF-2 α dephosphorylation⁵⁷ or 4) exploit host cellular factors in order to inactivate PKR⁵⁶.

Both ADAR p150 and PKR are IFN-inducible RNA-binding proteins. Their effects are entirely opposite and their alternation represents a fine balance between viral infection and host cell defense⁵⁴.

5.4. Epstein-Barr virus

Epstein-Barr virus belongs to Herpesviridae family of viruses⁵⁴ whose characteristic is the production of miRNA⁵⁸. It is a linear double-stranded DNA virus with envelope and icosahedric capsid and tegument containing viral proteins⁵⁴. It targets human B cells and epithelial cells. The life cycle of EBV consists of a lytic and a latent phase, for which three (I-III) types have been identified. During lytic infection, EBV causes infectious mononucleosis, while the latent phase is associated with Burkitt lymphoma, Hodgkin disease and some epithelial cancers⁵⁴.

To understand the effects of ADAR proteins on EBV infection it is necessary to understand the viral life cycle, its lytic and latent phase, and miRNA biogenesis. Transition between different stages of latency is regulated with viral transcripts and viral miRNAs (miRNA) respectively. An A-to-I editing of pri-miRNAs can suppress processing in two stages i) inhibition of Drosha in nucleus; ii) halting processing of pre-miRNA to miRNA by Dicer inhibition⁵⁹.

A study conducted by Izasa et al. reports miR-BART6-5p RNAs regulative role in EBV infection and latency. The study showed that A-to-I site specific editing in combination with deletion of three uracil residues in miRNA precursor completely inhibits Drosha with total loss of matching pre- and mature miRNA. Furthermore, site specific editing of pri-miR-BART6 as well as mutations found in latently EBV-infected cells by ADAR1 cause significant reduction in loading of miR-BART6-5p RNAs on the microRNA-induced silencing complex. Moreover, miR-BART6 controls viral proteins for transition from types I-II (less immunoreactive) to type III (more immunoreactive) latent state of EBV infection. In addition, miR-BART6 targets host-cell Dicer transcript and consequently downregulates the host miRNA involved in immune response as well as viral RNA transcripts⁵⁹.

Another study conducted by Lei et al. reports A-to-I editing events in miR-BART3 of EBV with pre-miR-BART3 being site-specifically edited at four sites. These editing events affect the targeting and biogenesis of mature

miR-BART3. Lastly, editing sites inside seed regions of miR-BART3 significantly reduces its silencing activity and even biogenesis⁶⁰.

Chao et al. discovered hyperediting events facilitated by ADAR in the lncRNA lytic transcripts derived from EBV latency origin of replication. These lncRNAs interact with a cell's innate immune system and enable the progression of a viral lytic cascade⁶¹.

5.5. Hepatitis C virus

The Hepatitis C virus (HCV) belongs to Flaviviridae family of viruses, genera Hepacivirus. It is a single-stranded positive sense RNA (ss(+)RNA) virus with envelope and icosahedric capsid⁵⁴. It is transmitted parentally, sexually and vertically⁶². The Hepatitis C virus is the main infectious cause of hepatocellular carcinoma, most common type of liver cancer. Furthermore, it causes both acute and chronic hepatitis. Worldwide, 71 million people suffer from chronic hepatitis, therefore it represents a significant public health issue⁶².

A study conducted in 2005 by Taylor et al. reported that ADAR1 has a limiting role in the replication of viral RNA. By transfecting Huh7 cells (hepatocyte derived cellular carcinoma cell line) with a HCV RNA replicon (subgenomic in vitro self-replicating HCV RNAs) and treating them with IFN- α , inhibition of both PKR and ADAR1 showed increased replicon expression. On the other hand, in cells expressing ADAR1, random A-to-I hyperediting events were observed leading to loss of HCV replicon⁶³. This research shows that ADAR1 in HCV infection has an antiviral role whilst preventing HCV replication. The mechanism itself is not yet clarified but this antiviral role could be due to induction of RNA degradation or mutations in viral RNA genome sequences, both of which are caused by hyperediting effect.

Conclusion

It is evident that ADAR proteins have many biological roles in pathogenesis of multiple human diseases. However, pattern that leads to pathogenesis is not uniform and underlying mechanism are poorly understood. In some cases, ADAR proteins are elevated while in others they are decreased. Identical model applies on ADAR mediated A-to-I editing events which range from hyperediting on Alu sequences to site-specific editing in GRIA2 Q/R site. In this review we discussed disruptions in cellular mechanisms caused by ADAR mediated A-to-I editing events and fine balance between innate immune responses. Possibility to edit dsRNA ensures variety of different pathogenesis mechanisms that we explained thoroughly in chronic myeloid leukemia, breast cancer, glioblastoma, mental illnesses, neurodegeneration and viral infection. In cancer, ADAR1 acts as oncogene and tumor suppressor while ADAR2 has neuroprotective role in brain cancers. Death of neurons through excitotoxicity seems to be evolutionary integrated negative feedback mechanism in case of ADAR2 site-specific editing failure. Furthermore, synaptic scaling as a direct consequence of ADAR2 editing CNS transcripts causes various mental illnesses. Both proviral and antiviral effect of ADAR proteins was noticed, depending highly on their role in the evasion of immune system. ADAR proteins as such represent a niche for further research and opportunity for novel treatment development in some diseases.

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Dorotea Neuberg

Nationality: Croatian

Phone: (+385) 0989796130

Date of birth: 29/12/1998

Gender: Female

Email address: doroty.n@gmail.com

Email address: dneuberg@student.uniri.hr

Address: Radnička 14a, 42220 Novi Marof (Croatia)

EDUCATION AND TRAINING

Bachelor degree in Biotechnology and Drug Research

Department of Biotechnology, University of Rijeka [10/2017 – 09/2020]

Address: Radmile Matejčić 2, 51 000 Rijeka (Croatia)

<https://www.biotech.uniri.hr/hr/>

Thesis: ADAR Proteins in Human Diseases

Immunology, Neuroimmunology, Chemoinformatics, General miRNA Biology, Biology of Mental Illness, Pharmacology, Biochemistry, Biochemistry, Advanced Microscopy in Neuroscience

High School Diploma

Prva gimnazija Varaždin [09/2013 – 06/2017]

Address: Petra Preradovića 14 , 42 000 Varaždin (Croatia)

<http://www.gimnazija-varazdin.skole.hr/>

Final grade: 4.97/5.0

- President of Student Council 2017.
- Drama group Theatron

WORK EXPERIENCE

CEO for One Month

The Adecco Group [06/2020 – 07/2020]

Address: Ul. fra Grge Tuškana 37, 10 000 Zagreb (Croatia) - <https://www.adeccogroup.com/>

City: Zagreb

Country: Croatia

Shadowing management of the Adecco Adriatic in their day-to-day tasks and responsibilities. Participating on the cluster and zone management meetings. Creating industry breach marketing strategy for implementation of HR solutions to banking and insurance companies in Croatia. Creating innovative employment solutions for underprivileged groups. Participating in competition for *Global CEO for One Month*.

President

Biotechnology Student Association at University of Rijeka [10/2019 – 07/2020]

City: Rijeka

Country: Croatia

Supervising and advising project managers and their work especially in financial and legal matters. Monitoring implementation of 12 projects on weekly basis, finding solutions for occurring problems. Assembling meetings and sessions, reporting annual financial report to Association's assembly. Raising funds through donations. Creating strategies for future development.

Organization Comitee Member

3rd Biotechnology Students Congress with International Collaboration - „In research we believe “ [02/2020 – 05/2020]

City: Zagreb

Country: Croatia

Inviting relevant speakers and ensuring sufficient funding through donations on behalf of USBRI which is partner organisation.

Project manager

Biotechnology Student Association at University of Rijeka [02/2018 – 01/2020]

City: Rijeka

Country: Croatia

Managing Conference "Future and perspective" in all segments: raising funds, inviting relevant speakers, finding venue, social media marketing, public promotion. Reshaping routine programme of Conference into three

interactive panels with relevant speakers from industry and science with addition of three workshops for improving students' soft skills. Main goal was increasing employability of biotechnology students. Coordinating members of organising board. Evaluating quality of programme through satisfaction pool. Moderating panel discussion with biotech companies.

Senior F2F Fundraiser

UNICEF [02/2018 – 11/2019]

City: Rijeka

Country: Croatia

Communication with citizens, presenting UNICEF's mission and action on local, national and international level. Sensitising of public and motivating for donations on monthly basis through standing orders.

Vice-President

Biotechnology Student Association at University of Rijeka [02/2018 – 10/2019]

City: Rijeka

Country: Croatia

Promoting Association on social media and representing it at public events (e.g. Student Day Festival, Scholarship fare...). Taking care of human resources – recruiting new members, coordinating volunteers and project managers. Dealing with financial matters and ensuring funding for projects.

Promotor

Yasenka [04/2019 – 07/2019]

City: Rijeka

Country: Croatia

Communication with citizens in pharmacies and presentation of Yasenka's pharmaceutical portfolio in order to increase the sale. Education of public about active substances. Arranging promotions with pharmacy staff on monthly basis.

Student Teaching Assistant at course Cellular and Molecular Biology

Department of Biotechnology, University of Rijeka [11/2018 – 01/2019]

City: Rijeka

Country: Croatia

Assisting professor on the course by helping students while they do practical work on microscopes and prepare sample.

VOLUNTEERING

Regional Coordinator at Croatian Association of High School Students

[Varaždin, 09/2016 – 10/2017]

Coordinating volunteers and organizing International student's week in Varaždin with events for high-school students (e.g. computer games tournament, parties, anti-corruption activities, student film festival).

Mentor at European Foundation for Philanthropy and Social Development

[Novi Marof, 12/2015 – 12/2016]

Advising and guiding local teams through 3 month long program of innovation and idea implementation in local communities. Guiding team through expansion of initial project idea and taking track of their progress.

Young Advisor to Ombudsman for Children, Republic of Croatia

[Zagreb , 09/2014 – 09/2017]

Advisor and associate to Office of Ombudsman for Children. Suggesting potential changes in Croatian laws concerning children's best interest. Making strategy against peer violence - "Stop the painful silence".

Student Representative in Board for Quality Control at Department of Biotechnology

[University of Rijeka, 10/2019 – Current]

Analysing student satisfaction survey results and suggesting solutions on students' behalf for occurring problems. Creating strategy for improving employability rate.

Student Representative in Student Council at Department of Biotechnology

[University of Rijeka, 10/2019 – Current]

Collaborating with department's administration and professors in improving overall quality of study programme. Representative of students in Department's Council. Advocating changes in policies which are in student's best interest in order to improve student standards.

CONFERENCES AND SEMINARS

European Forum Alpbach

[Alpbach, Austria , 15/08/2018 – 03/09/2018]

Scholarship holder awarded by EFA Foundation for three-week participation on interdisciplinary platform for science, politics, business and culture. Participation on two seminars and relevant Symposia: Political, Economy, Health and Technology. **Seminars:** Precision medicine and its impact on society, Ethics in action: Economics and sustainable development. **TU Austria Innovation Marathon:** 24h of design thinking in order to create innovative fridge for Liebherr company.

<https://www.alpbach.org/en/>

Global-In Fellowship

[Berlin, Germany, 07/2017 – 07/2017]

3-week summer program on diplomacy, international relations, entrepreneurship and global understanding. Workshops in field of economy, law, entrepreneurship and innovation. Enhancement of leadership abilities and rhetorical skills through MUN and simulation of political systems.

<https://fellowship.global-in.org/>

Erasmus+ Youth Exchange

- Transforming Europe: WWI and Its Consequences 1918-2018; Ikast, Denmark, 2016;
- History- youth's opportunity; Črnomelj, Slovenia, July 2016
- Outdoor activities across borders; Edsbyn, Sweden; August 2015

HONOURS AND AWARDS

Dean's Award

Department of Biotechnology [2019]

For excellent academic achievements and student activism.

STEM Scholarship

Ministry of Science and Education, Republic of Croatia [2020]

For excellent academic achievements in Science.

Award for the best graduate student in generation

Prva gimnazija Varaždin [2017]

For successes during entire high school education, engagement in promotion of schools reputation and organisation of activities for students.

Rotary Award for Excellence

Rotary club Varaždin 1181 [2016]

For achievements in formal high school education, extracurricular activities and engagement in local community.

Oskar of Knowledge

Croatian Agency for Education [2013]

For first place on National competition in biology

LANGUAGE SKILLS

Mother tongue(s):

Croatian

English

LISTENING: C2 READING: C2 WRITING: C2

SPOKEN PRODUCTION: C1

SPOKEN INTERACTION: C1

German

LISTENING: B1 READING: A2 WRITING: A2

SPOKEN PRODUCTION: A2 SPOKEN INTERACTION: A2

DIGITAL SKILLS

Outlook / Social Media / Microsoft Office