Berkeley Pharma Tech
Journal of Medicine

Activation Induced Deaminase and Potential Therapeutic Avenues

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Activation Induced Cytidine Deaminase

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Keywords:
AICDA
Lupus
Gene Therapy
Autoimmune
Cancer
Therapeutic Advances

Submitted April 29, 2022
Accepted June 24, 2022
Published July 19, 2022

Full Open Access

Abstract

Activation induced cytidine deaminase (AID) is an important enzyme that creates mutations in DNA via deamination of a cytosine base into a uracil. AID, also referred to as activation induced deaminase (AICDA), plays a crucial part in the human immune response as it is essential for isotype switching and cellular differentiation. However, aberrant expressions in some pathways has been implicated in a plethora of diseases. There is a pressing need for research and comparison of current literature that informs related therapies. Previous studies have explored potential mechanisms by which AID works and subsequently ways to target gene therapies based on this information. Due to AID's complexity, there have been many challenges along the path that led to our current understanding of the beneficial and harmful nature of AID. Furthermore, a better understanding of the way AID works can aid with the development of more efficacious therapies. Although further research on the topic and additional testing in humans and animal models are needed, it is clear that AID may play an important role in the development of therapeutic treatments in diseases like cancer, lupus, and type 1 diabetes.

Introduction

Previous studies and research have established that AID regulates secondary antibody diversification. There are many different immunoglobulin (Ig) diversification processes, such as somatic hypermutation (SHM), class switch recombination (CSR), and gene conversion (GC)[4]. SHM allows for B cells to diversify in order to respond to threats to the immune system,[5] while CSR allows for the generation of different classes of antibodies[5]. GC is a process in which mutations can occur in the antibody genes[5]. AID is central to CSR/SHM and plasma cell differentiation and is encoded by AICDA and B lymphocyte maturation protein 1 (Blimp-1) which is a transcription factor encoded by Prdm1[6]. AID and its transcription factors underpin Ab and autoantibody responses[7] . The deamination results in a change from a cytosine base to a uracil base in Ig genes, and this can result in either CSR or SHM, depending on the deoxyribonucleic acid (DNA) repair pathway. AID expression is upregulated by inflammatory cytokines like interferon-γ and tumor necrosis factor (TNF)-α which induces p53 mutations in inflammatory or cancer cells. Although AID is typically associated with and expressed in B-cells, it can also be expressed, for example, in embryonic germ cells or pluripotent cells like oocytes. AID proteins have been shown to be expressed during early B-cell development in both human fetal liver and adult bone marrow [9].

It is important to note that AID is a potent enzyme which instigates genomic diversity for both beneficial[10] and harmful outcomes in humans. This can best be depicted in Figure 1, which summarizes much of the following section. AID differs from other Apolipoprotein B mrRNA Editing Catalytic Polypeptides (APOBECs) specifically due to the size and orientation of its substrate specificity loop[12]. It has a larger loop that extends away from the active site and thus can accommodate two purines next to a target C[12]. Despite some sequence similarity to APOBEC cytidine deaminases, AID's critical function in Ab diversification in CSR cannot be substituted by other APOBEC proteins[10]. While aberrant deaminase activity can certainly threaten the genome, recent biotechnological efforts have focused on harnessing and targeting deaminase activity in base editors that are related to AID[11].

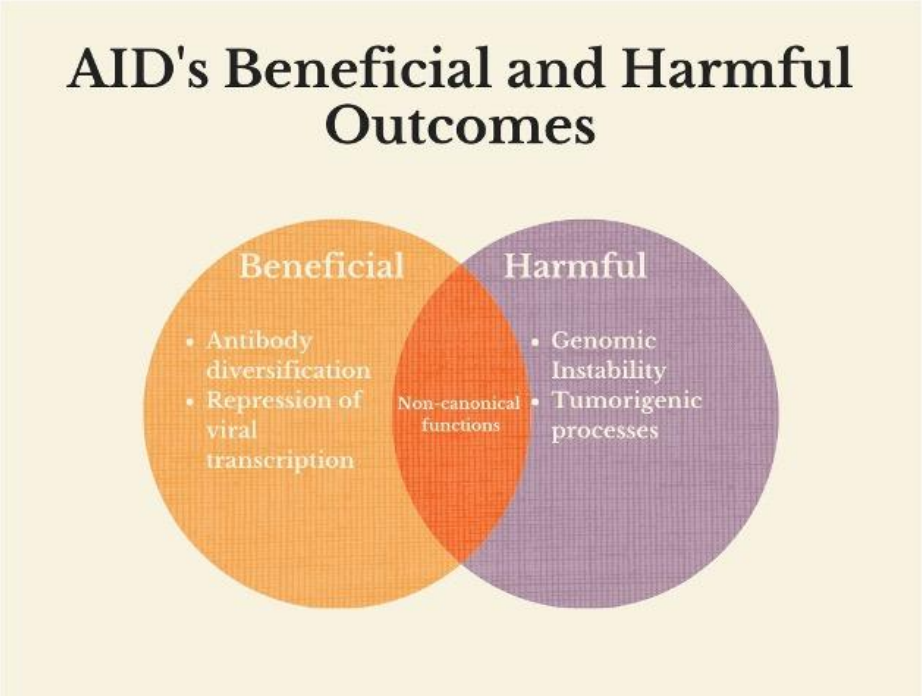

Figure 1: A diagram summarizing some of the beneficial and harmful outcomes of AID

AID, a potent DNA mutator, must be tightly regulated to prevent any off-target effects which can result in a plethora of problems including mutations in non-Ig genes, genomic instability, interchromosomal translocations, and cellular neoplastic transformation[13]. AID has previously been implicated in the tumorigenic process in B cell tumors potentially through the induction of chromosomal translocations and mutations in tumor suppressor genes and oncogenes[14]. AID expression has also been implicated in the pathogenesis of human B cell malignancies[15]. Indeed, accumulating evidence suggests AID is pro-oncogenic and induces cancer-promoting mutations or chromosomalrearrangements[16]. Another detrimental impact of AID is the generation of autoimmunity, which can occur after on-target point mutations in variable genes produce antibodies with high affinity for self-proteins[11]. These detrimental effects are important to consider when choosing to target AID in potential research projects. Other studies have proposed more novel functions for AID. For instance, one has suggested that AID functions as an adaptor protein that represses viral transcription, which would have implications for the development of anti-HIV therapeutics and other therapies [17]. Moreover, AID can exert non canonical functions when aberrantly expressed in epithelial cells and was

long known to lack specific inhibitors which prevented therapeutic applications to modulate AID functions[18].

Mechanism

Historically, the discovery of AID and its essential role in antibody diversification kindled a debate over AID's nucleic acid substrate, which inspired the DNA deamination model and identification of downstream players in the CSR pathway such as the DNA glycosylase UNG[19]. Subsequently, this discovery led to a race to uncover ssDNA as the target of AID's enzymatic activity and its dependence on transcription for deamination[19]. It is important to note that AID deaminates C→U only on ssDNA and does not function on dsDNA or RNA[12]. Figure 2 depicts this type of deamination reaction. Nearly two decades prior to this research, there was an observation that noncoding RNA transcripts originated from within the IgH locus and could be playing a role in CSR[19].

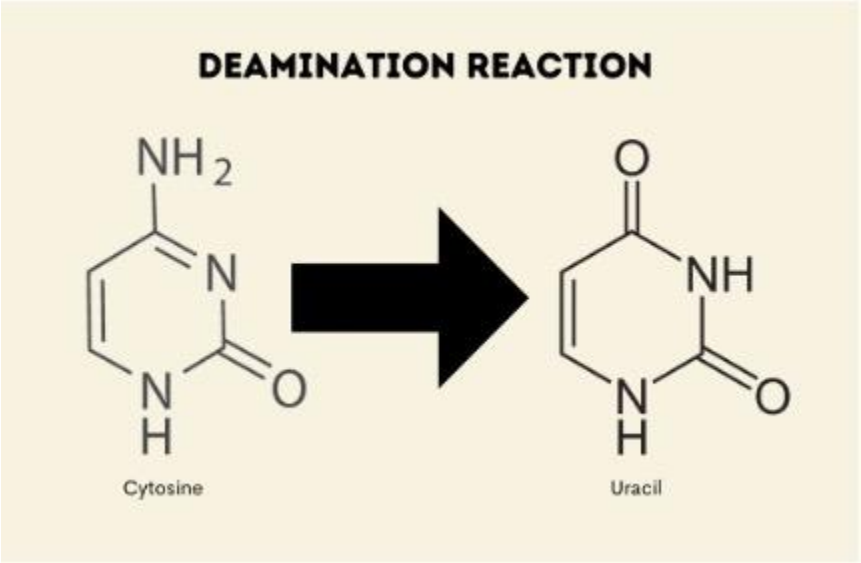

Figure 2: Cytosine to Uracil deamination reaction wherein an amine group is removed

SHM generates point mutations in the Ig variable regions while CSR exchanges the Ig heavy chain constant region, and ultimately this gives rise to antibodies with enhanced affinity and new effector functions[20]. AID initiates both these processes through deamination of cytosine to uracil in Ig variable and switch gene regions, and the resultingU:G mismatches are

subsequently processed by uracil-DNA glycosylase (UNG2)and a pathway that requires

The mechanism by which AID works has not been entirely discerned. Namely, the mechanism of AID targeting has especially been a long-standing mystery[10]. Currently, there are many different findings that are piecing together the puzzle of how exactly AID works. In terms of frequency, the number of molecules containing deamination in both DNA strands at the acceptor switch region corresponds to its class switch efficiency. It has been proposed that the minimal requirement for a DNA double-strand break (DSB)formation is as low as only one AID deamination event on both DNA strands[4]. There are also several proposed mechanisms for AID function. AID may target template and non-template strands at similar frequencies and predominantly after R-loops are processed by cellular enzymes that expose DNA on both DNA strands[4]. Additionally, AID footprints may be distributed evenly across the entire length of the S region, unlike SHM, which is not evenly distributed over a distance; thus AID deaminates S and V regions with distinct mechanisms. It has also been suggested that AID-mediated DNA demethylation occurs due to the deamination of methylated cytidine residues in single-stranded DNA, followed by DNA repair[7].

A long-standing hypothesis on AID targeting, known as the hotspot hypothesis, has recently been under re-evaluation. This hypothesis considered a short sequence motif (AGCT)conserved in all S regions as functionally important for CSR, proposing that it exerts its function via its overlapping AID hotspot structure[23]. However, an initial weakness of this theory was that these sequences are very common in the genome[23]. Another study determined one of the first crystal structures of maltose-binding protein (MBP)-fused AID and its complex with cytidine (C),deoxycytidine (dC),and deoxycytidine monophosphate (dCMP).These structures can help explain the discrimination between DNA and RNA in AID catalysis and reveal that AID has a bifurcated substrate-binding surface[10]. This supports the theory that one AID recognizes two adjacent ssDNA overhangs from one structured substrate to achieve high affinity[10]. G4 structured substrates induce AID cooperative

oligomerization, which could promote clustered mutations in the Ig S regions[10]. Overall, the bifurcated substrate binding surface and oligomerization interface are both an essential component of CSR and help elucidate recognition of structured substrates as an important AID-targeting mechanism, specifically in the Ig S regions[10]. It has therefore been suggested that G4 substrates mimicking Ig S regions are preferred AID targets in vitro. This recent finding is a departure from our previous understanding of AID targeting. This data also posits that AID preference for these substrates is likely due to their bundled ssDNA overhangs structure rather than the primary sequence motif, which was long believed to bewhy AID preferred these substrates[10]. It is important to recognize that a definitive complex structure with fully characterized substrate conformation is still lacking and must be developed[10].

Many proposed therapies suggest that selective inhibition of AID may ameliorate the conditions. Ultimately,further experimentation and analysis with more sensitive techniques that may eventually be developed is needed to more fully understand the mechanism of AID inhibition. Given that the crystal structure of AID has recently been resolved, future efforts would certainly benefit from structural modeling approaches[10]. A more definitive structure could serve as a template for potential therapeutic intervention against AID[10]. Progress on AID structure is very timely alongside the growing knowledge about Ig class switch region nucleic acid structures, which are supported by functional studies[24]. Already, we are seeing promising results from initiatives focusing on AID. Platforms like GENEVESTIGATOR consolidate publicly available studies from microarrays, mRNA sequencing, and more under healthy conditions versus diseased states[25]. Using these comparisons is one potential strategy for a comprehensive analysis of the role of AID in the pathobiology of immune- or inflammatory-based diseases and cancer[25]. It has also been suggested that we may eventually be able to analyze AID gene signatures to get decisive determinants of patient-specific or patient-group-specific antiviral response, which could allow us to understand how viruses can impact different individuals[25].

Estrogen and AID

Estrogen has been found to reverse the repression of AID, resulting in a subsequent boost in AID expression. This is proposed to occur through the upregulation of HoxC4, which, together with NF-κB, critically mediates AID promoter activation[6]. There may, however, be additional epigenetic mechanisms at play that serve to regulate AID expression. Estrogen reverses HDI-mediated inhibition of AID and CSR in Ab and autoantibody responses through the downregulation of B cell miR-26a, which targets AID mRNA's 3'UTR[6]. As epigenetic modifiers, SCFA HDIs, like miR-26a and miR-125a, inhibit AID expression and CSR through the upregulation of select B cell miRNAs, which silence AID[26]. This is interesting as it may provide an explanation for the female bias in autoantibody-mediated autoimmune diseases like lupus[2]. Yet, an experimental and fully functioning in vivo model of the human immune system is needed in order to understand the epigenetic mechanisms relating to the human Ab and autoantibody response[6].

Autoimmune Diseases

Cellular reprogramming, broadly, is a mechanism that must be further explored. Currently, there are three approaches to induce reprogramming: cell fusion, nuclear transfer, and iPSC[14]. Cell fusion is a great way to understand nuclear plasticity and is a main element of many cancer processes[14]. Nuclear transfer, more commonly referred to as cloning, has potential therapeutic applications, although ethical concerns exist[14]. iPSC technology is anexcellent option given that it has potential therapeutic applications for clinical use without ethical concerns and can be used to model human diseases and screen potential new treatments[27]. DNA methylation is a major barrier to induced pluripotent stem (iPS) cell reprogramming, and putative DNA demethylase protein AID can erase DNA methylation at pluripotency gene promoters, which will subsequently allow cellular reprogramming[14].

Autoimmune diseases are detrimental to the health and wellbeing of individuals globally.

One example of such a disease is common variable immunodeficiency (CVID), which is a primary immunodeficiency characterized by hypogammaglobulinemia and different degrees of B cell compartment alteration[28]. We found reduced Bcl-2 protein levels in memory B cells from CVID.

Hypertension is another medical condition where the study of AID can be useful. In the USA, nearly 50%of the adult population has hypertension, and prevalence increases to ~80%at advanced age[29]. B cell Ig production is dependent on a subset of B cells called GC B cells, which are dependent on AID and may play a causal role in the pathophysiology of hypertension. The GC reaction is driven by IL-21 and T follicular helper (Tfh) cells, which are transcription factors associated with AID and have been demonstrated to play a role in hypertension and hypertensive end-organ damage[30]. It is possible that B cells and Ig contribute to hypertension in specific cases as in autoimmune diseases or preeclampsia[31]. However, future studies should investigate inducible genetic B cell deletion in adult animals to determine if B cells are viable therapeutic targets for hypertension[31].

Multiple Sclerosis (MS) is another debilitating chronic disease. B cell depleting therapies are a potential way to ameliorate symptoms in MS given that B cells play a critical role in the MS disease process[32]. There is a presence of B cells in active lesions and the cerebrospinal fluid of MS patients[32]. In a recent study, the community was able toglean more information on the role of secondary diversity of the BCR in experimental autoimmune encephalomyelitis (EAE) and identify IgG class-switched B cells as potential therapeutic targets for the treatment of MS[32]. AID was also found to presumably still exert some subtle effect on rMOG-induced (myelin oligodendrocyte glycoprotein) disease trajectory[32].

Arthritis is a debilitating disease that can result in a lot of pain A potential novel treatment for inflammatory arthritis includes Fraxinellone[33]. The therapeutic effect of fraxinellone was associated with the inhibition of cellular differentiation and activation. It has been shown to attenuate the clinical and histologic features of inflammatory arthritis in mice[33]. There

was a lower expression of AID and Blimp-1 following treatment with Fraxinellone[33].

Remarkably, it also alleviated synovial inflammation and osteoclastogenesis in mice[33]. Other drugs such as belimumab, a targeted therapy approved for systemic lupus erythematosus (SLE), serve as examples of how targeted therapies that disrupt the AID pathway can be beneficial[34]. Further investigation is needed to see the side effects on normal cells.

Cancer

AID, as previously mentioned, has been largely suggested to induce cancer-promoting mutation. AID is expressed in more than 40% of primary human chronic lymphocytic leukemia (CLL) cases, but AID expression can be harnessed for antileukemic effect after inhibition of the RAD51 homologous recombination (HR) factor 4,4'-diisothiocyanatostilbene-2-2'-disulfonic acid (DIDS)[16]. This is a novel antineoplastic role of AID that can be triggered by inhibition of HR, which is a new paradigm to treat AID-expressing tumors and has had proof of principle studies conducted[16]. This treatment has also been suggested for use in type 1 diabetes[6]. Another avenue that has been considered is the chronic administration of HSP90inhibitors, which decreases AID protein levels and has been shown to reduce disease severity in a mouse model of acute B cell lymphoblastic leukemia in which AID accelerates disease progression[18]. This is promising, as a proof-of-concept study has been published that showed HSP90inhibitors directly target AID in vivo, and endogenous humanAID is sensitive to them [18]. Yet another study has suggested that targeting AID is beneficial in the immunotherapy ofAID positive tumors because siRNA silencing of AID in plasmacytoma dramatically increases its susceptibility to immunotherapy by cytotoxic T lymphocytes[15]. Overall, AID has shown to be a promising target in the aforementioned instances and more research may yield additional insights.

Conclusion

Although further research will help the scientific community to glean more clear insights, it is clear that elucidating how AID works will help with the development of novel therapeutic strategies for a multitude of diseases. Disrupting the AID pathway can have potential therapeutic effects. However, it is important to remain cognizant of the fact that AID is a complex component of the human immune system, which is in and of itself a complex system. With that in mind, therapeutic approaches targeting AID must undergo a variety of testing and considerations.

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