

DNA deaminases: AIDing hormones in immunity and cancer

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Abstract It is well established that hormones can cause cancer, much less known is how they induce this change in our somatic cells. This review highlights the recent finding that estrogen can exert its DNA-damaging potential by directly activating DNA deaminases. This recently discovered class of proteins deaminate cytosine to uracil in DNA, and are essential enzymes in the immune system. The enhanced production of a given DNA deaminase, induced by estrogen, can lead not only to a more active immune response, but also to an increase in mutations and oncogenic translocations. Identifying the direct molecular link between estrogen and a mutation event provides us with new targets for studying and possibly inhibiting the pathological side-effects of estrogen.

Keywords Hormones · DNA mutations · Cancer · AID · APOBEC3 · Autoimmunity

Introduction

In 1938, the first cheap synthetic estrogen-like chemical (diethylstilbestrol) [1] was made available to patients, first

as an experimental drug, then a few years later with FDA approval. In previous decades, the benefits of estrogen as a supposed remedy for a variety of diseases provided fertile ground for subsequent pharmaceutical exploitations. Yet, even as early as the late 1800s, clinicians observed a link between hormones and cancer [2, 3], and in 1932, controlled laboratory research showed that estrogen can induce breast cancers [4]. Surprisingly, over the last 75 years, and despite the subsequent tragedy that resulted from the diethylstilbestrol, the molecular mechanism behind how estrogen exerts its oncogenic effects remained mostly elusive. Tests, including the Ames mutagenesis assay, were unable to show any significant effect of estrogen as a DNA-damaging agent. This led to the prevailing conclusion that estrogen-induced cancer through its ability to activate cell proliferation. More recently, other theories have tried to explain the oncogenic potential of estrogen, they included: inhibition of apoptosis, inactivation of DNA repair, and radicals from oxidative stress or metabolic derivatives of estrogen (if present at high concentrations) forming DNA adducts (reviewed in [5–7]). Although each theory addresses a particular aspect of estrogen's oncogenic potential, none are fully satisfactory in explaining how physiological amounts of estrogen can be oncogenic.

In 2002, we discovered a novel molecular mechanism that explained how antibody genes were diversified after antigen encounter during a humoral immune response [8]. Although known to shuttle between the nucleus and the cytoplasm, activation-induced deaminase (AID) is a predominantly cytoplasmic enzyme that, upon activation, enters the nucleus and is targeted to the immunoglobulin (Ig) locus. The enzyme then deaminates cytosine bases (dC) to uracil (dU) in single-stranded DNA (Fig. 1A; for reviews please see [9–11]). The protein itself had been identified a few years earlier, as an essential gene for antibody

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diversification (i.e. class switching (CSR) and somatic hypermutation (SHM)), and was named AID [12, 13]. Usually the DNA lesion (either as dU or as a dU:dG mismatch) is faithfully repaired back to cytosine (Fig. 1B), but in the Ig locus it leads to DNA mutations and DNA recombination (Fig. 1C & D). During SHM, single point mutations are introduced into the DNA of the Ig locus, which manifest themselves as, and are selected for, amino acid changes in the antigen-binding site. Targeting the constant region of the Ig locus with AID during CSR, allows for homologous DNA regions to recombine and loop out the intervening sequence, thereby placing a different constant domain into the active Ig locus. This change allows for the antibody to obtain a different effector function (e.g., from a predominantly membrane-bound IgM to a soluble IgE). The hallmark for both events is a natural protein-inducing DNA damage that does not lead to DNA repair, but to DNA alterations in somatic cells.

Although a number of AID's functions are unique to antibody diversification, further analysis has shown that our genome contains a family of DNA deaminases, some of which are active anti-viral agents—the AID evolutionary descendant APOBEC3 family of proteins [14–16]. APOBEC3G's DNA deamination activity is known to inhibit HIV replication, thought to be by inactivating the first-strand cDNA of the HIV genome during viral replication.

The discovery of DNA deaminases showed for the first time, that naturally occurring unaltered enzymes could mutate the DNA of somatic cells. This was in direct contrast to the dogma that somatic cells should maintain a stable genome, and made these enzymes obvious candidate oncogenes. AID's oncogenicity was demonstrated when overexpression of AID in transgenic mice caused tumor formation in different tissues (lung [17], lymphatic [17], and liver [18]). More recently, it was shown that AID was required for the *c-myc*/IgH translocations of Burkitt lymphomas [19–21]. As shown in Fig. 1E, during this translocation *c-myc*'s promoter recombines with the Ig locus, causing oncogenic expression of the *c-myc* gene. Aside from *c-myc* [22], it has been known that the process of SHM (or AID) targeting can occur outside the Ig locus [23, 24], leading to DNA-deaminase-induced mutation in tumor suppressors or proto-oncogenes [25]. Importantly though, the oncogenicity and DNA mutability of the DNA deaminases are not due to a genetic defect in the proteins, but rather a consequence of their normal (albeit mis-regulated) function. With the potential to mutate our genomes on a daily basis, understanding how AID is regulated will have a direct impact on immunity, immune pathologies, and cancer.

In our recent publication [26], we were able show that estrogen could stimulate the production of AID in primary B cells. Although primary mature B cells are differentiated

and the AID locus may be 'available' (in terms of chromatin status), we did not require any co-stimulatory signals to detect the estrogen enhancement. We also confirmed that the increased production in mRNA showed a concomitant increase in AID protein. Furthermore, we observed a direct correlation with estrogen treatment, AID mRNA production and AID function in Ig class switching, and somatic hypermutation. Interestingly, the most significant correlation occurred between estrogen treatment and the number of potentially oncogenic *c-myc*/Ig translocations. We also observed this estrogen-induced response with most of the APOBEC3s, indicating that the response to hormones is ancient and conserved.

In terms of molecular mechanisms, we were able to demonstrate that the cellular estrogen receptor (ER) is bound to the AID promoter after estrogen treatment. This seems to recruit a transcription activator complex, and thereby drive the transcription of the AID gene. Interestingly, when we co-stimulated the cells both with estrogen and through the NF κ B pathway, we observed an enhanced binding of NF κ B to the promoter (known to activate AID mRNA production [27]), and enhanced AID mRNA production, which was greater than from either stimulus alone. This may have immune consequences, in that during an immune reaction, additional stimulatory signals provided by estrogen would enhance AID production, leading to a potential hyperactive humoral immune response.

There is a large body of evidence that females can have a more pronounced immune reaction upon infection, which has also been observed at the level of Ig production [28, 29]. This gender bias is also seen in immune pathologies that are dependent on antibodies, such as systemic lupus erythematosus (SLE) [30, 31], an autoimmune disease that is ten times more prevalent in women than in men. Therefore, the ability of estrogen to enhance or even hyper-activate AID production, could partially explain the molecular mechanisms of certain autoimmune pathologies, via AID's role in antibody maturation.

Although men have estrogen as part of their endocrine system, the levels are much reduced compared to women. In terms of overall immune-fitness though, there does not appear to be a strong bias of men vs. women. Why then, is a potentially highly oncogenic protein under the control of such a panpleiotropic hormone? One can only speculate on the evolutionary origins, but a plausible hypothesis is that AID (or its ancestor) was a genome guardian against foreign DNA (as mentioned in [8, 10, 14]). The enzyme could act on a mobile DNA element and inactivate it through deamination/mutation. This is partially substantiated by the following observations: (1) AID can partially inhibit retroviral infections [32]; (2) the APOBEC3 proteins predominately act to inhibit retroviral infections and retroelement mobility [14, 15, 33]; and (3) the Ig gene and its recombination potential

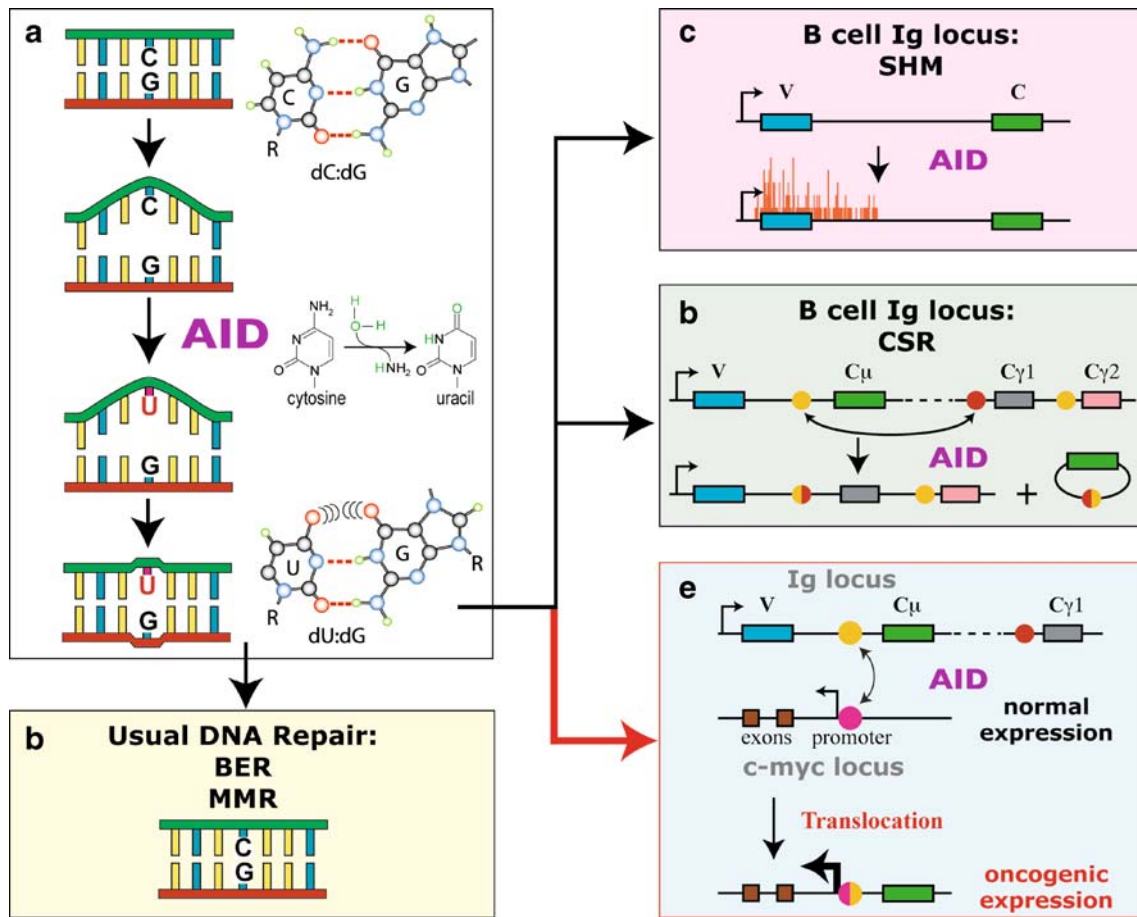


Fig. 1 The function and consequence of AID. (A) AID uses hydrolytic deamination to form uracil in single-stranded DNA, thereby inducing a twofold lesion in the DNA, an unusual base and a base mismatch. (B) Normally, various DNA repair pathways repair the

lesion back to cytosine. (C) and (D) At the immunoglobulin locus, the AID-induced lesion leads to SHM and CSR. (E) AID can induce oncogenic translocations during CSR, if the c-myc promoter rearranges into the Ig locus rather than another Ig constant region

was evolutionary derived from a retroelement [34, 35]—thereby linking the activity of AID to an invading DNA. As a genome guardian, it would be advantageous to have expression during the most vulnerable stage for retroelement infection of development—the unfertilized oocyte [36]. Already 5 years ago, we were able to show that AID is expressed in isolated mouse oocytes and embryonic germ cells [37]. In our current work [26], estrogen was able to increase AID mRNA in ovarian-derived primary cells by over 20-fold. This provides good evidence that AID-induced deamination is not limited to B cells. In line with a more global response of AID expression due to estrogen, analysis of cell lines derived from breast, ovarian, placental, and prostate tissue, also showed that AID was upregulated in response to estrogen. Analogously, the other DNA deaminases (APOBEC3 members) were also activated in various tissues and cell lines after estrogen treatment.

It is not fully understood how AID, or its evolutionary descendants, can inactivate foreign DNA. Aside from

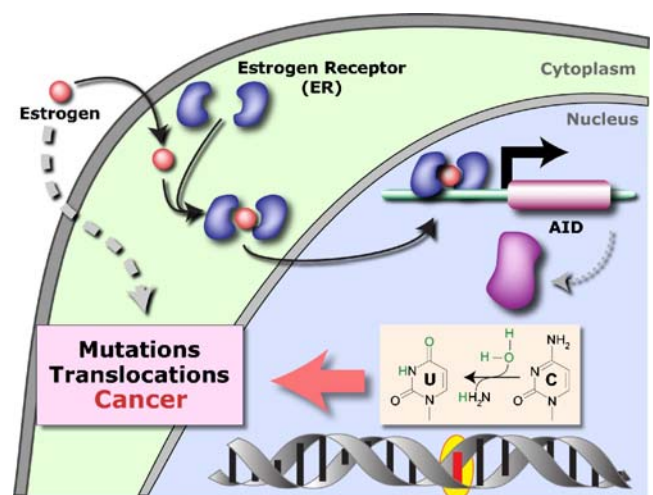


Fig. 2 Model of how estrogen induces DNA damage. Estrogen enters the cell and is bound by the ER. The dimeric ER-estrogen complex enters the nucleus and induces AID expression. This leads to an increase in mutations and translocations, and potentially cancer

mutating cytosines in mobile elements in our genomes, an intriguing additional possibility is via deamination of 5-methyl-cytosine (5meC). 5meCs in the context of CpGs usually inactivate the mobility of DNA elements [36], but some elements are resistant to this mark. We have already demonstrated that AID can deaminate 5meCpG [37], and recent genetic evidence has linked AID to CpG DNA demethylation [38]. It is therefore possible that estrogen-driven mutation of CpGs during oncogenesis may also be due to aberrant AID expression.

After more than a century, our current model (Fig. 2) may have brought us a step closer in understanding how estrogen can directly induce mutations leading to cancer in higher vertebrates. We suggest that endogenous or exogenous estrogen is absorbed by the target cell. In the cytoplasm, the dormant estrogen receptor (ER) will bind to the ligand and dimerizes. These structural alterations allow the receptor–ligand complex to enter the nucleus and target estrogen responsive genes. At the promoter of the DNA deaminase gene AID, the ER–estrogen complex recruits proteins for mRNA transcription. The enhanced AID message is then translated into increased amounts of AID protein. Excessive AID enters the nucleus and begins to deaminate cytosine bases to uracil. If these DNA lesions are not repaired, the consequence is mutation and translocations—leading to autoimmunity or oncogenesis. As the model predicts, the activity of estrogen is not dependent on cell replication, occurs in cells that are estrogen responsive, and does not require any a priori genetic mutations for manifestation.

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