APOBECs and Virus Restriction

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The apolipoprotein B mRNA editing enzyme, catalytic polypeptide (APOBEC) enzyme family in humans has 11 members with diverse functions in metabolism and immunity. The enzymes deaminate cytosine in RNA or single-stranded (ss) DNA, which forms uracil. The name is derived from the first discovered family member, APOBEC1, that edits the apolipoprotein B mRNA and other mRNAs. Uracil in RNA has a coding function, but in single-stranded (ss)DNA, it is promutagenic. Amazingly, these modification enzymes make cellular function and immunity better. For example, some family members purposefully induce these mutations in viral genomes to restrict their replication. However, events can sometimes go wrong, leading to inappropriate expression or activity, which can result in somatic mutations and cancer evolution.

Keywords: APOBECs ; HIV ; restriction factor ; mutagenesis ; anti-viral

1.Introduction

The APOBEC family is divided into subfamilies that include APOBEC1, Activation Induced cytidine Deaminase (AID), APOBEC2, APOBEC4, and the APOBEC3 enzymes that are found in placental mammals [1]. The Special Issue articles focus on the ability of APOBEC3 enzymes to inhibit a diverse number of viruses and act as a cross-species barrier to viruses, APOBEC3 polymorphisms, and functions of specific APOBEC3 enzymes (https://www.mdpi.com/journal/viruses/special_issues/APOBECs_viruses). The articles primarily focus on how APOBEC3 enzymes inhibit human immunodeficiency virus type 1 (HIV-1), which is the virus most studied for susceptibility to APOBEC3 anti-viral activity. In this context, APOBEC3 enzymes are part of another larger family of enzymes, termed host restriction factors. It has been 19 years since the discovery of APOBEC3G-mediated restriction of HIV-1 [2]. Shortly thereafter, activities from multiple APOBEC3 family members against HIV and other viruses were discovered. There are still new discoveries being made in the APOBEC3 field, which this Special Issue summarizes in eight review articles and two research articles.

2. In humans

In humans, *APOBEC3* genes are all on human Chromosome 22 and have duplicated from a single APOBEC3 gene found in placental mammals, such as mice, to seven in humans (named APOBEC3A to H, excluding E) [3]. Uriu et al. detail this evolution and discuss how the birth of the flag-ship APOBEC3, APOBEC3G, was formed [4]. The gene first occurred in Simiiformes but not in prosimians [4]. Since the birth of the *APOBEC3G* gene coincides with the invasion of endogenous retroviruses (ERVs), the evolutionary data provide strong evidence that the original function of APOBEC3G was to suppress these ERVs [4].

To inhibit HIV, the APOBEC3 enzymes must become encapsidated into the budding virion [5][3][6]. This enables access to the viral genome and newly synthesized (-) DNA during reverse transcription. The APOBEC3 enzymes deaminate cytosine to form uracil when the (-) DNA is single-stranded after RNaseH degradation and before (+)DNA synthesis. This results in uracil templating the addition of adenine upon synthesis of the (+)DNA, resulting in a hypermutated and likely inactivated virus. APOBEC3s can also physically block reverse transcriptase, which prevents full proviral DNA synthesis [5]. HIV produces a protein called Vif which tries to prevent APOBEC3 encapsidation by multiple mechanisms. Stupfler et al. describe how Vif uses multiple ways to block APOBEC3G activity [6]. Vif is a thermodynamically unstable protein since it is composed mainly of loops, without a stable core structure. For stability, Vif uses binding partners. The main one is the co-transcription factor CBF-β. By CBF-β binding to Vif, it is relocalized to the cytoplasm, altering the transcription profile of the cell, which includes decreasing transcription of APOBEC3G [6]. Furthermore, Vif can bind to the 5′UTR of A3G mRNA and impair translation by 70–75%. Stupfler et al. discuss how two stem-loops, SL2–SL3, in the 5′UTR are specifically bound by Vif and cause ribosome stalling [6]. Vif can also inhibit packaging of APOBEC3G through a physical interaction. Finally, the most well-known method is through Vif mediating the degradation of APOBEC3 enzymes through ubiquitination and proteasomal degradation. Vif acts as the substrate receptor in a Cullin5 ubiquitin ligase complex [6].

Nonetheless, some APOBEC3G can still escape. If Vif also becomes encapsidated, then Vif can inhibit APOBEC3G catalytic activity [6], but this does not appear to happen for other APOBEC3s, such as APOBEC3H [7]. This multifunctional role of Vif for inhibiting APOBEC3s is fascinating in itself, and Vif additionally inhibits cell cycle progression of infected cells [8].

3.In mice

In mice, the APOBEC3 (mAPOBEC3) is different from humans when restricting retroviruses. First, mice have only one *APOBEC3* gene [9]. Second, Salas-Briceno et al. describe how mouse retroviruses such as mouse mammary tumor virus (MMTV) and several strains of murine leukemia virus (MLV) are inhibited by a deamination-independent mechanism, which likely involves mAPOBEC3 binding the reverse transcriptase to block polymerase activity and increase reverse transcriptase insertion errors [9]. Additionally, during MLV infection, the mAPOBEC3 can bind directly to the protease-gag-polymerase (PR180gag-pol) precursor and perturb its autocleavage [9]. There are few to no deaminations recovered from mouse retroviruses exposed to mAPOBEC3, even with robust restriction of replication [9]. The deamination-independent mechanism has often been questioned with HIV restriction since human APOBEC3s also have strong deamination-dependent activity. However, the results in mice demonstrate how this mechanism can also be powerful. The MLV encodes an alternate glycosylated form of the Gag polyprotein that stabilizes the viral core and blocks mAPOBEC3 access to the reverse transcriptase complex [9]. The MMTV can evolve resistance to mAPOBEC3 by increasing the processivity of the reverse transcriptase [9].

4. APOBEC3 enzymes and HIV

For the APOBEC3 enzymes that restrict replication of HIV, APOBEC3G, APOBEC3F, APOBEC3H, APOBEC3D, and APOBEC3C, there are numerous polymorphisms [3]. Polymorphisms often, but not always, occur at the interaction site of the viral antagonist protein and are useful for counteracting virus suppression mechanisms [10]. Although these polymorphisms have a role in battling present day HIV infection, the origins date back to the transmission of HIV into humans from simian immunodeficiency viruses (SIV) in other primates. Uriu et al. and Gaba et al. describe how HIV was transmitted into humans [5][4]. HIV-1 is a transmission from chimpanzee or gorilla [5][4]. HIV-2 is a transmission from sooty mangabey monkeys [5][4]. Since HIV causes a lifelong infection and has been infecting primate species for millions of years, the host-pathogen interaction is highly specific. The Vif of one SIV can induce the degradation of that host's APOBEC3 enzymes, but not other hosts [5][4]. Thus, APOBEC3 enzymes (and other restriction factors) act as cross-species barriers. Gaba et al. details how the present day amino acid sequences of HIV-1 or HIV-2 Vif were formed from adaptations made to overcome the species barrier [5]. This is also reflected in the polymorphisms of the APOBEC3 enzymes [5]. Host populations that maintain multiple polymorphisms can potentially thwart infection by "surprising" the Vif with an APOBEC3 for which it cannot induce degradation [5].

An important feature of most APOBEC3 enzymes that restrict HIV is their ability to multimerize, either by self-association or with RNA [11]. Multimerization on RNA promotes encapsidation of APOBEC3s into HIV virions, and for APOBEC3H specifically, dimerization with RNA is an inherent part of the enzyme structure [11]. The nature of this multimerization has been debated in the literature. Chen describes how structural studies have been able to detail these specific interactions [11]. The full-length structure of rhesus macaque APOBEC3G showed that dimerization through N-terminal domains creates an interface for RNA to bind [11].

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