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ISG20, an actor of the innate immune response

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Abstract

The interferon (IFN) system is a major effector of the innate immunity that allows time for the subsequent establishment of an adaptive immune response against wide-range pathogens. The effectiveness of IFN to control initial infection requires the cooperation between several pathways induced in the target cells. Recent studies that highlight the implication of the 3′–5′ exonuclease ISG20 (IFN Stimulated Gene product of 20 kDa) in the host's defenses against pathogens are summarised in this review.

Keywords: Interferon; Exonuclease; ISG20; Innate immune response

1. Introduction

Cellular and extracellular ribonucleases appear to be major contributors to the protection against various pathogens including viruses and bacteria. RNase L, the first discovered IFN-activated RNase (reviewed by C. Bisbal and R.H. Silverman in this issue) is a dormant cytosolic endoribonuclease that is activated by short oligoadenylates produced by the 2′–5′ oligoadenylate synthetase following viral infection or double-stranded RNA treatment. Degradation of viral RNAs and cleavage of cellular 18S and 28S rRNAs by the activated-RNase L lead to the inhibition of protein synthesis, thus preventing viral propagation. Since then, some other ribonucleases have been suspected or demonstrated to be involved in antiviral defenses. In particular, RNases targeting the dsRNA formed in almost all viral infections represent good candidates for ribonuclease-mediated antiviral effects. Indeed, dsRNA duplexes can be hyper-edited by members of the adenosine deaminase enzyme family (ADA) resulting in up to 50% adenosine to inosine conversion and a specific cleavage by a cytoplasmic endoribonuclease that requires a RNA structure fitted to hyper-edited RNA [1,2]. This process provides an efficient mechanism to remove long uninterrupted dsRNAs associated with virus infection, suggesting that the IFN-inducible cytoplasmic isoform of ADA (ADAR1) represents a new pathway in the IFN-mediated antiviral action. In addition, adenosine to inosine editing dramatically changes the stability of dsRNA structures, resulting in a stronger vulnerability to attacks by single-stranded RNases [3]. In particular, inosine-containing single-stranded RNA and unwinding dsRNA edited by ADAR1 have been reported to be highly degraded by a specific 3′–5′ exonuclease termed I-RNase [4]. Some extracellular ribonucleases also display antiviral properties. The eosinophil-derived neurotoxin protein (EDN or RNase2) and the eosinophil cationic protein (ECP or RNase3), members of the RNase A family found in secretory granules of human eosinophilic leukocytes, reduce the infectivity of certain RNA viruses including respiratory syncytial virus (RSV) [5] and HIV [6]. In the same way, human onconase has been shown to act as a ribonuclease-dependent antiviral agent [7].

2. ISG20, a 3′–5′ exonuclease induced by interferon

The human ISG20 gene (IFN Stimulated Gene product of 20 kDa) was first identified based on its increased expression in response to IFN treatment [8–10]. Its modulation by type I and type II IFNs is under the control of the transcription

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factors IRF-1 on a unique Interferon Stimulated Response Element (ISRE) [10–12]. In the mean time, ISG20 was independently identified by its induction in response to estrogen hormone and termed HEM45 (human estrogen regulated transcript) [13]. Amino acid sequence analysis underlines that the encoded ISG20 protein is a member of the DEDDh subgroup of the DEDD exonuclease superfamily, which includes RNases such as RNase T and D, the proofreading domains of the Pol I family of DNA polymerases, and other DNA exonucleases [14,15]. This superfamily is characterized by a large structurally related exonuclease domain of about 150 amino acids (EXO III domain) in which homologies are distributed at three distinct exonuclease motifs termed Exo I, Exo II, and Exo III defined by four conserved acidic amino acids, three aspartate (D) and one glutamate (E) and a conserved histidine (h) residues (Fig. 1). Members of this superfamily can have both RNase and DNase activities and require two divalent metal ions for catalytic activity. The crystal structure of ISG20 reveals an exonuclease domain very similar to those of the corresponding domains of two DEDDh DNases, the ε subunit of *Escherichia coli* DNA polymerase III and *E. coli* exonuclease I, suggesting that they could follow the same catalytic mechanism [16]. However, ISG20 recognizes the sugar moiety of the nucleotide in a slightly different manner from the two DNases. Indeed, the presence of two distinctive residues, Met14 and Arg53 in the active site of ISG20, that accommodate hydrogen bonds of the 2′-OH group of the UMP ribose may reflect for the preference of ISG20 for RNA substrates [16]. Accordingly, biochemical analysis has confirmed ISG20 as a 3′–5′ exonuclease with a preference for single stranded RNA over single stranded DNA [17]. The presence of a stem-loop structure at the 3′ end of RNA substrates causes a strong reduction in ISG20 RNase activity indicating that ISG20 operates poorly on double-stranded regions [17]. Like some members of DEDD superfamily recombinant ISG20 possesses a weak single stranded DNase activity. However, the exact *in vivo* contribution of this activity is not established. The observation that the substitution of a single conserved aspartic acid by a glycine residue is sufficient to abolish its exonuclease activity demonstrates that ISG20 is functionally related to the DEDD superfamily [17]. Interestingly, ISG20 appears solely composed of a unique exonuclease catalytic domain without apparent regulatory domain (Fig. 1) suggesting that the regulation of ISG20 activity may require complex interactions with other factors leading to a local and specific activation preventing non-specific cell toxicity. Indeed, RNases are typically present in very low amount in cells associated with a specific inhibitor or present in an inactive latent form requiring the presence of a specific activator. This is the case for RNase L whose activation is dependent on the presence of oligoadenylates synthesized by the 2′–5′ oligoadenylate synthetase in response to replicating dsRNA forms of viruses. The fact that overexpression of exogenous ISG20 appears detrimental for cell survival highlights the necessity of a strict control of its activity.

3. ISG20 expression in response to pathogen infections

Several studies, using microarray approaches strongly suggest that ISG20 may be a major effector of innate immune response against various pathogens including viruses, bacteria and parasites (Table 1). For example, ISG20 is induced in the liver of acutely hepatitis B virus-infected Chimpanzee during viral clearance, reflecting the impact of an adaptive T cell response that inhibits viral replication and kills infected cells [18]. During human immunodeficiency virus type 1 (HIV-1) infection, ISG20 expression is rapidly and strongly induced [19,20]. This induction seems to be dependent on the viral transcription activator Tat in immature human dendritic cells [21]. Interestingly, ISG20 induction was observed both in HIV-1 and adeno-Tat infections, in absence of detectable IFNs in the culture supernatants suggesting that its overexpression can occur directly through activation of cellular and viral transcription factors. In contrast, in human postmitotic neuron-derivative cell line, NT2-N, the induction of ISG20 expression by rabies virus (RABV) infection seems to be mediated through the production of β-IFN [22]. The involvement of IFN secretion in the induction of ISG20 observed during infection by other pathogens remains to be determined.

On the other hand, ISG20 expression appears strongly induced, independently of IFN secretion, through the activation of members of the interleukine-1/Toll receptor super-family (Toll-like receptor) by different pathogen-associated-molecular-patterns (PAMPs) known to exert a strong adjuvant effect on immune responses such as, oligodeoxynucleotides containing unmethylated CpG motifs [23], bacterial lipopolysaccharides [24,25], double stranded RNAs [12] and small interfering RNAs [26,27]. In this later case, the effect seems to be mediated both by IFN induction through the activation of the dsRNA-dependent protein kinase PKR, and independently of an IFN response by the activation of the transcription factor interferon-regulatory factor 3 (IRF3) [27].

Infection leads to activation of the TLRs, which in turn initiate intracellular signaling cascades that culminate in the activation of NF-κB/Rel family transcription factors. Thus, NF-κB has often been referred to as a central mediator of the immune response [28]. Interestingly, we demonstrated that ISG20 is induced by dsRNA using a sequential process involving, first the NF-κB transcription factors family, and later, the IRF-1 transcription factor [12]. On the basis of these

Fig. 1. Schematic representation of ISG20. The large EXO III structurally related exonuclease domain is represented in green. The conserved acidic amino acids aspartate (D) and glutamate (E) as well as the conserved histidine residue (h) characteristic of the DEDDh superfamily are in red. The three exonuclease motifs Exo I, Exo II and Exo III are boxed in blue.