The role of USP18 in interferon signaling and inflammation

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Innate immune response provides the host with an early protection barrier against invading pathogens and helps shape the nature and quality of the subsequent adaptive immune response of the host. Ubiquitin-specific protease 18 (USP18) plays an important role in the host innate immune response. This role is mediated both via its enzymatic and non-enzymatic functions. First, conjugation of ISG15 to its targets is counteracted by the enzymatic activity of USP18. Second, the C terminus of USP18 can bind to the type I interferon receptor subunit (IFNAR2) and compete with JAK1 receptor binding which inhibits the activation of the receptor associated kinases JAK1 and TYK2. While USP18 deficiency prolonged phosphorylation of STATs and increased ISG expression in response to IFN, forced expression of either wild type or enzymatically inactive mutatant USP18 in the cells reduces the phosphorylation level of STATs and the downstream transcriptional events. USP18 knockout mice have a greater resistance to viral/bacterial infection in several experimental mouse models. In addition, USP18 was found to inhibit IFN-α signaling but did not inhibit IFN-β, IFN-γ, IL-6, or IL-12 signaling pathways. Results from our previous study demonstrated that USP18 was overexpressed in the liver biopsy specimens of HCV patients who do not respond to the combination therapy of pegylated interferon-alpha (IFN-α) and ribavirin (RBV). We also found that increased expression of either wild type USP18 or catalytically inactive mutant USP18 blunted the effect of both type I and type III IFNs and promoteded HCV production in the J6/JFH1 culture system. Another study suggested that HBV infection is more rapidly cleared if USP18 expression level is reduced. Most recently it has been shown that USP18 may play an important role in mediating inflammation. Inhibition of USP18 leads to upregulation of several pro-inflammatory chemokine, including CCL5, CXCL10 and IL-15, via induction of the STAT signaling pathway and increases IFN induced beta cell apoptosis. Taken together, small molecule inhibitors against USP18 might be a viable approach to potentiate the host innate immunity leading to a better control of viral infections.

Keywords: USP18; innate immunity; inflammation

1. Introduction

USP18, also known as UBP43 due to its approximately molecular mass of 43 kDa, is a member of ubiquitin-specific proteases (USP) family [1]. USP family of deubiquitinating enzymes plays an essential role in numerous cellular processes. USPs remove ubiquitin from ubiquitinated protein substrates through the deubiquitinating enzymes [2]. The family members include more than 100 proteins which differ in size and amino acid sequence, however, all share significant homology around the cysterine residues (Cys domain) and histidine residues (His domain) that are required for catalytic activity of the enzyme [3]. In 1999, Liu et al. used PCR-coupled subtractive screening-representational difference analysis to clone USP18 from AML1-ETO knockin mice, and they named the gene as UBP43 [1]. Later this gene was also cloned from respiratory syndrome virus (PRRSV)- infected porcine lung macrophages [4], a murine N1E-115 cell line transfected with RNase-L [5], and a human melanoma cell line treated with a combination of fibroblast interferon (IFN-β) and the protein kinase C activator mezerein (MEZ) [6]. These results demonstrated that USP18 is inducible by IFN and viral infection, indicating that USP18 might be playing an important role in the host innate immune response and inflammation.

2. USP18 suppresses IFN signaling pathway

Many studies clearly demonstrated that USP18 plays an important role in type I interferon signal pathway. Interferons (IFNs) were first described in 1957 as glycoproteins with strong antiviral activities that represent one of the first lines of host defense against virus or microbial infections [7]. Viruses or bacteria infection can induce IFN expression. IFNs are broadly classified into three types: Type I, II and III, based on the structure of their receptors on the cell membrane surface [8]. Due to their ability to modulate immune responses, they have been considered as treatment option for autoimmune diseases and several cancers [9,10]. Type I IFNs have been used as the standard of care (SOC) for Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) infections [11]. Type I IFNs belong to the class II family of a-helical cytokines, which are composed of IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω [12]. IFN-α and IFN-β binds to the same cell surface IFNα/β receptor (IFNAR) and activates tyrosine kinases Janus kinase 1(JAK1) and tyrosine kinase 2 (TYK2), which then phosphorylates the signal transducer and activator of transcription (STAT) family of proteins. Tyrosine-phosphorylated STAT1 and STAT2 migrate to the nucleus, where they recruit IFN-regulatory factor 9 (IRF9) to form the ISG factor 3 (ISGF3) complex. ISGF3 binds to the promoter region of IFN-stimulated response elements

(ISREs) in Interferon Stimulated Genes (ISGs) to promote transcription of ISGs, resulting in the upregulation of a few hundred ISG proteins, many of which have direct anti-viral activities [13,14].

Several mechanisms have been demonstrated to be able to attenuate IFN-stimulated Jak/Stat signaling [15], one of which is USP18 [16]. There is a lack of USP18 results in strengthened and prolonged STAT1 phosphorylation together with increased expression of a few hundred ISGs [17]. As the result, USP18 knockout (USP18−/−) in mice leads to IFN hypersensitivity, prolonged JAK-STAT signaling, and increased resistance to the cytopathic effects caused by some viruses including Sindbis virus (SNV), vesicular stomatitis virus (VSV), and lymphocytic choriomeningitis virus (LCMV) [18]. USP18 inhibits type I IFN signaling through a direct interaction between USP18 and the IFNAR2 subunit of type I IFN receptor [16]. The interaction between endogenous and exogenous USP18 and IFNAR2 in vivo blocks the interaction between JAK and the IFN receptor, thereby reduces the phosphorylation of the receptor and STATs and suppresses signal pathway and downstream biological responses. Since Type I IFNs are widely used as antiviral agents in the therapy of chronic viral infections, such as HCV and HBV [19], USP18 inhibitors may represent a promising strategy to enhance the anti-viral activity of type I IFNs.

3. Enzymatic functions of USP18

Interferon stimulated gene 15(ISG15) and USP18 are among the most abundant ISGs induced by type I IFNs or by viral infections. The ISG15/USP18 pathway is important for the host innate immune response to viral infections such as (HCV) [20]. ISG15 was the first ubiquitin-like protein modifier identified. As in ubiquitination, ISG15 can conjugate to potentially hundreds of target proteins (ISGylation) through the sequential enzymatic action of E1 activating enzyme (Ube1L), E2 conjugating enzyme (Ubc8), and some E3 ligases [21-23]. Due to the fact that ISG15 is strongly expressed after type I interferon stimulation and ISG15 conjugates to a broad panel of target proteins, some of which are involved in innate immunity [24], it has been the focus of attention as an important regulator of the host immune response and has been shown to play a protective role in a number of viral infections [25]. A number of studies demonstrated that ISG15 has antiviral activity in many cells and tissue culture under conditions of ISG15 overexpression or siRNA knockdown [26,27]. Numerous viruses have been reported to be inhibited by ISG15 in vitro [25], such as Influenza virus, vesicular stomatitis virus (VSV), human papilloma virus (HPV), avian sarcoma leucosis virus (ASLV), human immunodeficiency virus 1 (HIV-1) [28], Ebola virus-like particles (VLPs), West Nile virus (WNV) , dengue virus, Sendai virus(SeV), Japanese encephalitis virus (JEV), Newcastle disease virus (NDV), and vaccinia virus. USP18 is the major ISG15-speicifc protease that stripes ISG15 from its target proteins [18]. As a result, decreased USP18 expression leads to increased ISGylation.

4. Role of USP18 in human chronic hepatitis virus infections: HBV and HCV

It is likely that USP18 plays an important role in suppressing the effects of exogenous IFN-α treatment in chronic HCV infection. Pegylated interferon (IFN) plus ribavirin is the standard treatment of chronic hepatitis C virus infecton but has many unpleasant side effects. We previously observed differential gene expression levels between 15 nonresponder, 16 responder, and 20 normal liver biopsy specimens. We identified 18 genes whose expression levels differed significantly between all responders and all nonresponders ($P < .005$). The expression of USP18 is increased in the nonresponders [29]. And increased expression of USP18 in pretreatment liver tissues of patients chronically infected with HCV predicts treatment nonresponse [30]. These studies indicated that increased expression of USP18 in the liver blunted the effect of exogenous IFN-α. To further explore the role of USP18 in IFN resistance in HCV infection, Randall et al. found that knockdown of USP18 can prolong the activation of JAK/STAT signaling and increase IFN anti-HCV activity by 40-100 folds [17]. We recently also demonstrated that overexpression of either wild type USP18 or non-enzymatic mutant USP18 blunted the effect of both type I and type III IFN and stimulated HCV production in the J6/JFH1 HCV culture system. Taken all these results together, increased expression of USP18 not only promotes HCV production but also inhibits IFN anti-HCV activity, leading to persistent infection of HCV.

Hepatitis B virus (HBV) infection is one of the major causes of liver diseases. Although a vaccine is available, hepatitis B remains a major health problem in many countries. HBV is an enveloped-, partially double-stranded DNA virus [31]. Chronic HBV infection is one of the major causes of liver cirrhosis and hepatocellular carcinoma [32,33]. Currently, there are several drugs targeting viral replication have been approved by FDA for clinical use. Pegylated interferon (IFN) plus ribavirin is also the standard treatment for chronic hepatitis B. Studies show that USP18 plays important role in the innate immune response to HBV infection. Kim et al. found that decreased expression of USP18 not only suppressed HBV replication but also accelerated the clearance of HBV infection [34]. Using a mouse model of acute HBV infection by a replication competent DNA injection [35,36], they compared the level of HBV DNA between wild type and USP18−/− mice and they found HBV DNA, was strongly reduced in the USP18 -/- mice. This result is in line with the reports that USP18−/− mice showed increased resistance to some virus infections [18,34]. In addition, knockdown of USP18 decreased the level of HBV DNA with increased level of some ISG mRNAs. Interestingly, ISGylation does not affect the replication of HBV. Taken these results together, reduced expression of USP18 facilitates

HBV clearance. This effect may be due to the fact that inhibition of USP18 expression leads to hypersensitivity to IFN. In other words, decreased level of USP18 results in a strengthened immune response.

5. USP18 in beta cell death and inflammation

Type 1 diabetes (T1D) is a chronic autoimmune disease targeting pancreatic beta cells. Studies showed that IFN may initiate and accelerate of the autoimmune process in T1D by inducing direct beta cell apoptosis [12,37]. There is a positive correlation between elevated IFN-α level in blood and T1D associated with enterovirus infections [38]. The key factor STAT1 of IFN signaling is also playing an important role in modulation of beta cell inflammation and death. Knockout of STAT1 gene in the non-obese diabetic mice inhibit the development of type 1 diabetes. These results suggest that IFN signaling may trigger and amplify the local inflammation to modulate beta cell death. It has been shown that USP18 plays an important role in type I IFN-induced beta cell inflammation and apoptosis [39,40]. Santin et al reported that knockdown of USP18 induced inflammation and IFN-induced beta cell apoptosis through increased type I IFN signaling and mitochondrial pathway of cell death. They demonstrated that inhibition of USP18 induces pro-inflammation chemokine production; including CCL5, CXCL10 and IL-15, by amplifying the STAT signaling pathway. USP18 knockout increases the expression of T1D candidate gene MDA5 (melanoma differentiation-associated protein 5). This suggests a cross talk between a candidate gene for T1D and the type I IFN signaling pathway to increase pro-inflammatory responses in beta cells. These date collectively demonstrated that USP18 is a key regulator in beta cell inflammation and death.

6. Conclusion

Interferon induces increased expression of ISGs through different signaling pathways; some ISGs play a potential role in cellular defenses against viral infections and inflammation. Many IFN-induced proteins directly interact with viral or host proteins, by inhibiting viral transcription, degradation of viral RNA, inhibition of protein translation to suppress viral replication and packaging. However, the detailed mechanism of these antiviral proteins needs further elaboration; especially the molecular mechanisms of USP18 mediated innate immune response and interferon resistance of HBV and HCV. In view of the interferon is widely used in clinical treatment for various viral infections, a deeper understanding of ISG functions will help develop more effective treatment strategies.

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