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Review

Versatility of USP18 in physiology and pathophysiology

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Ubiquitin-specific peptidase 18 (USP18) is a multifunctional protein and its roles are still being investigated. This enzyme removes ubiquitin-like molecules from their substrates and the only known interferon-stimulated gene 15 (ISG15) specific protease. Apart from its enzymatic function, it also inhibits interferon type I and III signalling pathways. USP18 is known to regulate multiple processes, such as: cell cycle, cell signalling and response to viral and bacterial infections. Moreover, it contributes to the development of several autoimmune diseases and carcinogenesis, and recently was described as a cardiac remodelling inhibitor. This review summarizes the current knowledge on USP18 functions, highlighting its contribution to the development of heart failure, given the fact that this disease's etiology is now considered to be inflammatory in nature.

Key words: peptidase, deISGylation, interferon, cardiac remodelling, heart failure, biomarker

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Abbreviations: DCs, dendritic cells; IFN, interferon; IFNAR, interferon type I receptor; ISG15, interferon-stimulated gene 15; ISRE, interferon-stimulated response element; JAK1, Janus kinase 1; JAK-STAT, Janus-activated kinase/signal transducer and activator of transcription; MAPKs, mitogen-activated protein kinases; ROS, reactive oxygen species; STING, stimulator of interferon genes; TNF-α, tumour necrosis factor alpha; T1D, type 1 diabetes; TYK2, tyrosine kinase 2; Ubls, ubiquitin-like molecules; USP18, Ubiquitin-specific peptidase 18; UBPs, ubiquitin-specific proteases

INTRODUCTION

Ubiquitin-specific peptidase 18 (USP18) is a cysteine protease of a dual nature. As an enzyme, it cleaves ubiquitin-like molecules (Ubls) from their target proteins, while as a substrate for an interferon (IFN) receptor it acts as an IFN type I and III signalling suppressor (Hoeller *et al.*, 2006; Manini at al., 2013; Hong *et al.*, 2014; Ketscher & Knobeloch, 2015; Honke *et al.*, 2016; MacParland *et al.*, 2016; An *et al.*, 2017; Basters *et al.*, 2017; Mustachio *et al.*, 2017; Basters *et al.*, 2018; Shaabani *et al.*, 2018; Gu *et al.*, 2019). These diverse functions make USP18 an interesting object for research and many studies describing this unique molecule have been published recently. Among others, its contribution to cell signalling, response to viral and bacterial infections, development of several malignancies and development of autoimmune diseases have been described (Liu *et al.*, 1999; Ritchie *et al.*, 2004; Hoeller *et al.*, 2006; Catic *et al.*, 2007; Duex & Sorkin, 2009; François-Newton *et al.*, 2011; Murray *et al.*, 2011; Burkart *et al.*, 2012; Guo *et al.*, 2012; Yim *et al.*, 2012; Coit *et al.*, 2013; Honke *et al.*, 2013; Hong *et al.*, 2014; Zhang *et al.*, 2015; Honke *et al.*, 2016; Shaabani *et al.*, 2016; Ying *et al.*, 2016; Zhang at al., 2016; An *et al.*, 2017; Arimoto *et al.*, 2017; Basters *et al.*, 2017; Mustachio *et al.*, 2017; Basters *et al.*, 2018; Shaabani *et al.*, 2018; Gu *et al.*, 2019). USP18 was also found to serve as an inhibitor of cardiac remodelling (Ying *et al.*, 2016). This review summarizes the abovementioned functions, emphasizing the role of USP18 as a biomarker with potential in cardiovascular disease monitoring.

CHARACTERISTICS

The USP18 protein belongs to a large family of ubiquitin-specific proteases (UBPs). Initially, the Usp18 gene was called Ubp43 since it encodes a 43-kDa protein with UBP properties, i.e. it cleaves ubiquitin or Ubls from their target molecules (Honke *et al.*, 2016). Initially, USP18 was cloned from mice expressing leukemia-fusion protein AML1-ETO and, afterwards, from virus-infected porcine alveolar macrophages and human melanoma cell lines (Honke *et al.*, 2016; Gu *et al.*, 2019).

Human USP18 has two isoforms which differ in the N-terminal region of the polypeptide chain. Both isoforms are localized in the cell cytoplasm and one of them, USP18-sf, is also detected in the nucleus. This is the main nuclear ISG15-specific protease, which suggests that USP18-sf may exert additional, more specific functions (Burkart *et al.*, 2012; Honke *et al.*, 2016). High expression of USP18 was detected in the liver, thymus and spleen. Lower amounts were found in the bone marrow, lungs and adipose tissue. Synthesis of USP18 is strongly induced by IFNs I and III, viral infections, lipopolysaccharide, tumour necrosis factor alpha (TNF-α) and genotoxic stress (Honke *et al.*, 2016).

USP18 presents the same three-dimensional structure as other USPs. It consists of three catalytic domains, described as: the finger, the palm and the thumb. The area between the thumb and palm domains contains a catalytic triad composed of a cysteine, an aspartate and a histidine residue. Unlike other USPs, this catalytic core is the only protein interaction domain in the USP18 structure (Basters *et al.*, 2018).

USP18 is the only known protease which specifically deconjugates interferon-stimulated gene 15 (ISG15), one of the Ubls, from modified proteins *in vivo*. According to some reports, under specific circumstances USP18 also acts as an ubiquitin protease. DeISGylation, which is analogous to deubiquitination, was shown to influence antiviral response mechanisms, genome stability and carcinogenesis. Similarly to USP18, ISG15 synthesis is induced by IFN-I, especially during viral and bacterial infections (Zhang & Zhang, 2011). The role of this protein is not fully understood, but it is known that ISGylation of viral proteins inhibits replication of many virus-

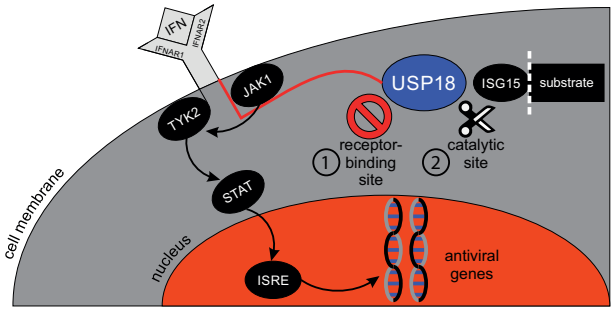

Figure 1. Schematic presentation of two types of USP18 activity: 1) IFN signalling pathway inhibition through binding with IFNAR2 subunit of type I IFN receptor and blocking activation of JAK-STAT cascade; 2) enzymatic deISGylating function.
Abbreviations: IFN, interferon; IFNAR1, first subunit of type I interferon receptor; IFNAR2, second subunit of type I interferon receptor; ISG15, interferon-stimulated gene 15; ISRE, interferon-stimulated response element; JAK1, Janus kinase 1; STAT, signal transducer and activator of transcription; TYK2, tyrosine kinase 2; USP18, ubiquitin-specific peptidase 18 (for details see text).

es. Free ISG15 was also described as an antiviral factor (Zhao *et al*., 2013).

Another USP18 role seems to be structurally separated from its enzymatic function. It attenuates type I IFN signalling by interaction with the IFNAR2 subunit of the IFN-I receptor (IFNAR) (Basters *et al*., 2017; Basters *et al*., 2018). As a result, it inhibits the Janus-activated kinase/signal transducer and activator of transcription (JAK-STAT) pathway, acting in a negative feedback loop (see Fig. 1). Type I IFNs are well-known for their antiviral, antineoplastic and immunomodulatory effects, which makes USP18 a factor that facilitates viral infections and that may promote carcinogenesis, but also prevents INF hypersensitivity and autoimmune disease development under physiological conditions (Arimoto *et al*., 2017). Recent studies described this activity as protease-independent. USP-18 knock-out mice, which presented an increased IFN response and died after administration of the IFN-inducing agent poly I:C, were saved by an enzymatically inactive USP18. Moreover, IFN hypersensitivity and interferonopathy were not observed in mice with USP18 inactivation that was limited to its catalytic properties (Basters *et al*., 2018).

INFECTIONS

USP18 is known for its proviral effect, which is exerted by a negative regulation of the IFN-I signalling pathway. IFN-I binds to IFNAR on the cell surface, which due to its kinase activity triggers Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) activation, which then initiates STATs phosphorylation. STATs activate the interferon-stimulated response element (ISRE) in the nucleus which, in turn, starts antiviral gene transcription. USP18 binds with IFNAR2, one of the two IFNAR subunits, and prevents JAK1 phosphorylation (Honke *et al*., 2016; Basters *et al*., 2017; Gu *et al*., 2019). Apart from IFNs, other factors, such as TNF-α or LPS, can also boost USP18 expression, reduce antiviral defence and facilitate opportunistic viral infections. Upon absence of USP18, phosphorylation of STATs is enhanced and there is an increase in expression of genes which encode antiviral proteins, cytokines, chemokines and genes for proteins that contribute to antigen presentation. Such an effect was described for viruses, such as: LCMV, VSV, HBV, HCV, HIV, Coronavirus, Sindbis, influenza B virus and the Herpes virus. Isg15-deficient mice do not present enhanced susceptibility to viruses, so this activity is not associated with the ISGylation process. Nevertheless, USP18's influence on antiviral mechanisms is ambiguous. Although IFN-I limits viral spread in the organism, it diminishes presentation of viral antigens to immune cells, which impairs the adaptive immune system response. Without efficient antigen presentation, the immune reaction is impaired and this can lead to the autoimmune process (Honke *et al*., 2016).

Another issue is USP18's complicated impact on antibacterial mechanisms. It has been demonstrated that Usp18 knock-out mice are characterized by less-pronounced growth of *Salmonella typhimurium* than the control animals. In contrast, mice with a missense mutation in USP18 ($Usp18_{lty9}$) present lethal susceptibility to *S. typhimurium* (Honke *et al*., 2016). According to Shaabani *et al*., IFN-I signalling attenuates the immune response in the course of several primary bacterial infections and promotes a secondary bacterial invasion following viral infections. Lack of IFN-I signalling leads to a reduction in bacterial replication and survival. Interestingly, it has been demonstrated that USP18 is responsible for the harmful effect of IFN-I during *Listeria monocytogenes* or *Staphylococcus aureus* infection of mice. The mechanism of this phenomenon is different than those described above. In this case, the authors suggest that USP18 inhibits the antibacterial TNF-α signalling and, as a result, reduces reactive oxygen species (ROS) production and facilitates proliferation of bacteria. IFNAR1 or Usp18 knock-out CD11c-Cre+ cells were observed to mitigate bacterial titres in several organs and to prolong survival. This phenomenon was proven to be independent of the IFNAR2 binding and isopeptidase domains (Shaabani *et al*., 2018). It is possible that USP18 may also involve other USPs and suppress TNF-α in different pathways. Such a relationship was described for USP20 which deubiquitinates mitochondrial adaptor protein STING (stimulator of interferon genes) (Zhang *et al*., 2016; Shaabani *et al*., 2018). However, other authors have shown that this recruitment, leading to STING stabilization, sustains cellular antiviral responses and decreases susceptibility to HSV-1 (Zhang *et al*., 2016). Without a doubt, further studies are needed to define this effect in more detail.

CARCINOGENESIS

Several studies have been published on the role of USP18 in carcinogenesis. Their diverse results suggest that USP18 can act both, as an oncogene and tumour suppressor, and that the ultimate effect depends on the tissue type and cellular background (Mustachio *et al*., 2017).

USP18's oncogenic role was defined for several types of tumours. Mustachio and others (Mustachio *et al*., 2017) had shown that expression of USP18 in the lung cancer tissue is higher than in a normal lung. Likewise, loss of USP18 expression led to reduced lung cancer cell growth, induced apoptosis and increased cell response to treatment. That and other studies revealed that USP18 directly stabilizes cyclin D1 and KRAS proto-oncogene GTPase, which are over-expressed or continuously activated in the lung cancer cells (Hoeller *et al*., 2006; Guo *et al*., 2012; Mustachio *et al*., 2017). Similar effects were described for the breast cancer cell line MCF-7 and glioblastoma cells (Honke *et al*., 2016). According to Guo and others (Guo *et al*., 2012) acute promyelocytic leukemia cells also present increased levels of USP18 and a

gain of USP18 expression stabilized the oncogenic protein PML/RARα in these cells (Ritchie *et al*., 2004). In turn, Duex and Sorkin had stated that due to Usp18 knock-out, EGFR expression was reduced up to 50–80% in several cell lines. Since this effect was assigned to EGFR mRNA translation inhibition, USP18 contribution to squamous cell carcinoma development is also possible (Duex & Sorkin, 2009).

On the contrary, Hong and others (Hong *et al*., 2014) had found an opposite role of USP18 in the context of cancer development. They stated that after induction by INF-γ in tumour cells, USP18 inhibits carcinogenesis and enhances anti-neoplastic immunity. Moreover, it not only modifies malignant cells, but also the tumour microenvironment, by regulating the synthesis of IFN-γ and activation of antigen-specific cytotoxic T lymphocytes (Hong *et al*., 2014; Honke *et al*., 2016). Subsequent studies may elucidate and highlight this controversial nature of USP18.

AUTOIMMUNE DISEASES

Another group of pathologies influenced by the USP18 and IFN-I pathway are autoimmune diseases. Type 1 diabetes (T1D) is a well-described example. A crucial role in its pathogenesis is played by dendritic cells (DCs), which are known for their considerable USP18 expression. In terms of a viral infection, when viral antigens are homologous to epitopes of the β islet cells, DCs present autoantigen to autoreactive CD8+ cells, which induce islet damage. It was shown in mice that the depletion of DCs diminishes viral replication and autoantigen presentation, which inhibits T1D development. This effect is thought to be explained by augmented IFN-I signalling and effective antiviral defence, resulting from suppression of USP18. Therefore, USP18 increases viral replication in DCs and can trigger an autoimmune reaction (Honke *et al*., 2013; Honke *et al*., 2016). On the contrary, other studies revealed that sufficient USP18 synthesis in β islet cells is mandatory to prevent them from autoimmune damage and the development of T1D. Increased expression of USP18 inhibits insulitis by limiting synthesis of proinflammatory chemokines. Moreover, USP18 influences the mitochondrial pathway of cell apoptosis and prevents IFN-induced β cell apoptosis (Santin *et al*., 2012; Honke *et al*., 2016).

Coit and others (Coit *et al*., 2013) had suggested that USP18 participates in pathogenesis of lupus. They noted a substantial hypomethylation of genes regulated by IFN in naïve T cells obtained from lupus patients; this included, among others, the Usp18 gene. These genes appeared to be over-expressed in all CD4+ T cells. Based on these findings, the authors hypothesised IFN-I hyperresponsiveness in lupus T cells.

Furthermore, some studies suggest that USP18 is crucial for the balanced function of microglia in the central nervous system. Without USP18, STATs activation is prolonged, which may lead to microgliopathy. USP18 may also be involved in pathogenesis of multiple sclerosis (Honke *et al*., 2016).

CARDIAC REMODELLING

Ying and others (Ying *et al*., 2016) had shed a new light on activity of USP18 and extended its action to the cardiovascular system. Their study demonstrated that USP18 inhibits cardiac remodelling and protects from the development of heart failure. In hearts of patients with dilated cardiomyopathy, USP18 expression was significantly higher than in hearts from healthy donors. Moreover, in murine hearts exposed to increased afterload, augmented expression of USP18 in cardiomyocytes was related to less pronounced myocardial fibrosis, reduced left ventricular wall hypertrophy and delayed onset of heart failure (Ying *et al*., 2016). In contrast, USP18-deficient mice presented advanced cardiac remodelling (Ying *et al*., 2016). According to Ying and others (Ying *et al*., 2016) cardioprotective effect of USP18 is a consequence of the TAK1-p38-/JNK1/2 signalling pathway inhibition, which is an example of MAPKs (mitogen-activated protein kinases) – dependent pathway (Ying *et al*., 2016). MAPKs are involved in myocardial hypertrophy and heart failure pathogenesis, since upon prolonged pressure overload they contribute to structural modification of the contractile proteins (Dick & Epelman, 2016). Our unpublished data indicate that the serum USP18 level is up to 10-fold higher in drug-naïve patients with early stage systolic-diastolic arterial hypertension than in normotensive subjects, and also greater than in patients with isolated diastolic hypertension. It is known that inflammatory processes take part in the development of heart failure and contribute to cardiac injury and repair (Dick & Epelman, 2016). Considering the above-mentioned facts, USP18 may indicate a counter-regulatory regenerative process to increased afterload, aiming at maintaining the proper cardiomyocyte structure and contractile function. In these terms, elevated serum USP18 level may result from intensified synthesis and/or cardiomyocyte cellular membrane damage and release into serum. If this thesis is true, it might be used as a cardiac remodelling biomarker in the future.

CONCLUSIONS

USP18 is a multifunctional protein which exerts both, enzymatic and IFN-I suppressing functions. Due to this multi-faceted nature, USP18 participates in several intra- and extracellular pathways, response to viral and bacterial infections, oncogenesis and autoimmune disease development. Although its role is not fully understood, recent studies suggest that USP18 may also serve as a cardiac remodelling biomarker. Expression of USP18 is significantly higher in damaged cardiomyocytes than in healthy cells. Moreover, it suppresses hypertrophy and fibrosis of the cells and delays the onset of heart failure in a murine model. Since cardiac diseases are now considered inflammatory in nature, the role of USP18 in their pathogenesis is very likely. Further studies are needed to resolve this issue.

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