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Ubiquitin-Specific Peptidase 18: A Multifaceted Protein Participating in Breast Cancer

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Abstract: Mammary tumors display high molecular heterogeneity with regards to their transcriptomes and proteomes. The regulation of several posttranslational modifications, such as ubiquitination and ISGylation, directly influences the proteome of breast cancer cells. In particular, ISGylation is emerging as a critical factor in different cancer types and is particularly relevant in breast cancer. This modification involves the covalent binding of interferon stimulated gene 15 (ISG15) to its target proteins.

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Interestingly, ubiquitin-specific protease 18 (USP18), also called UBP43, reverses ISGylation. In addition to its activity as a de-ISGylation enzyme, USP18 is also a negative regulator of type I IFN signaling. Several studies indicate a central role of USP18 in the pathogenesis of breast cancer. This chapter discusses recent insights gained in the molecular mechanisms of USP18 in breast cancer, and its potential implications for the development of novel therapeutic strategies for this disease.

Keywords: breast cancer proteome; ISGylation in breast cancer; IFN-I signaling in breast cancer; ubiquitin-specific protease 18 in breast cancer; USP18

INTRODUCTION

Breast cancer is a malignant mammary neoplasm that poses a serious health issue worldwide. The heterogeneity of breast cancer makes it difficult to develop novel strategies for the prevention, detection, and control of its progression. Most breast cancer cases are sporadic. Histologically, more than 80% of the cases develop in the mammary ducts with invasive potential and are known as invasive ductal carcinomas. At the molecular level, nearly 70% of breast cancer cases express estrogen receptor alpha (ERα) and are referred to as breast cancer ERα+ cases. The remaining 30% of cases do not express ERα, and are referred to as breast cancer ERα- cases (1, 2). The 13th St. Gallen International Breast Cancer Conference recommends a classification that includes luminal A, luminal B, HER2 overexpressing, and triple-negative subtypes (3). The main difference is that ERα is only detected in luminal A and B cancer types, but luminal A types are also characterized by PR ≥20% and Ki67 <20%; whereas luminal B-like types are characterized by PR <20% and/or HER2+ and/or Ki67 ≥20%. The type characterized by an overexpression of HER2 comprises nearly 15-20% of all cases of breast cancer. The last type is triple-negative, with a lack of ERα, PR, and HER2 expression, and is highly aggressive (3, 4).

These subgroups warrant different therapeutic strategies. For example, HER2-overexpressing breast cancer warrants therapies targeting HER2, a transmembrane receptor with tyrosine kinase activity that lacks a ligand-binding domain but can generate homodimers or heterodimers with other members of the epidermal growth factor receptor (EGFR) family. Therapies for HER2-overexpressing breast cancer therefore include antibodies (i.e., trastuzumab), inhibitors of the kinase activity of HER2 (i.e., lapatinib), and antibody-drug conjugates (ADC), which are cytotoxins covalently associated with monoclonal antibodies against HER2 (5). For luminal breast cancer, the commonly used therapy is endocrine therapy involving selective estrogen receptor downregulators (SERDs), selective estrogen receptor modulators (SERMs), and aromatase inhibitors (AIs) (6, 7). SERDs and SERMs target ERα, which is a transcription factor activated by 17 beta-estradiol that promotes the expression of pro-tumor genes in breast cancer. Because of either *de novo* or acquired resistance to these endocrine therapies (8), the generation of new target molecules for treatment is required. Several alterations associated with posttranslational modifications have been reported in the proteome of breast cancer cells. In particular, alterations in ISGylation and ubiquitination of proteins have been associated with this pathology. Interestingly, the reversion of these modifications can be modulated by ubiquitin-specific protease 18 (USP18), a de-ISGylase and deubiquitinase that is also deregulated in breast cancer. In this chapter, we discuss the relevance of USP18 and its functions in breast cancer.

GENERALITIES OF UBIQUITIN SPECIFIC PEPTIDASE 18 (USP18)

Many cellular processes are modulated by different post-translational modifications. One of these modifications is ubiquitination, which involves the covalent binding of ubiquitin to target proteins. This process can be reversed by deubiquitinase enzymes, which are central to its regulation. Moreover, ubiquitin-like proteins also participate in post-translational modifications via mechanisms similar to ubiquitination (9, 10). Ubiquitin-specific peptidase 18 (USP18), also known as ISG43, UBP43, and PTORCH2, is a member of the USP family that deubiquitinates proteins. Furthermore, USP18 enzyme is the only protease that has been reported to reverse ISGylation, and this ability is known as de-ISGylation (11–13). Additionally, USP18 has a negative effect on type I interferon signaling pathway (14, 15). Thus, USP18 is an ISG15 isopeptidase and a negative regulator of interferon signaling (Figure 1).

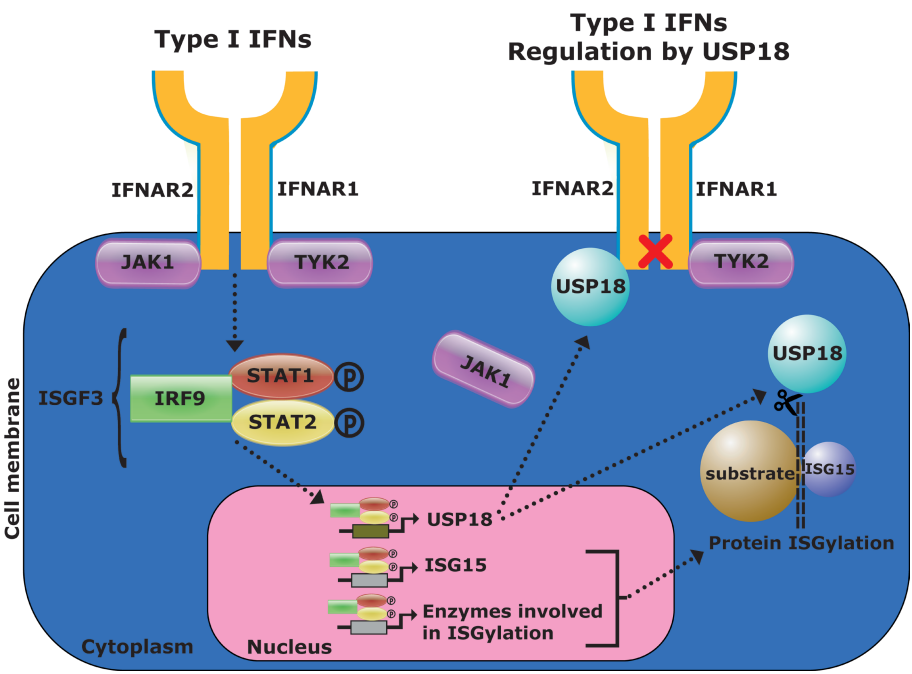

Figure 1. Molecular actions of ubiquitin-specific protease 18 (USP18) protein. Type I interferons (IFN-I) bind to its receptor formed by the heterodimer IFNAR1/IFNAR2 which is associated with Janus kinase 1 (JAK1) and Tyrosin kinase 2 (TYK2), triggering this signaling pathway. Genes that encode enzymes involved in ISGylation, interferon-stimulated gene 15 (*ISG15*) and *USP18,* are induced by IFN-I. USP18 associates with subunit 2 of IFN receptor (IFNRA2), inhibiting the binding of Janus-activated kinase 1 (JAK1). Consequently, USP18 inhibits the phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2.

USP18: from gene to protein and biology

The *Usp18* gene is located on mouse chromosome 6, whereas the human *USP18* gene is located on chromosome 22. This gene consists of 11 exons that encode a 43-kDa protein homologous to ubiquitin-specific proteases (UBPs). It has been reported that the expression of *Usp18/USP18* gene is increased by several inducers, such as type I and II interferons (IFNs) (12, 15–17), poly I:C (18), lipopolysaccharides (LPS) (18, 19), tumor necrosis factor-alpha (TNFα (19), and bacterial and viral infections (17, 20, 21). Interestingly, the promoter of this gene contains IFN-sensitive response elements (ISREs), which mediate the induction of *USP18* expression in response to IFNs (14, 15). High expression of *Usp18* has been found in the liver, spleen, and thymus, and is remarkably expressed in various lymphatic and hematopoietic cell populations, such as macrophages and T and B cells (22).

USP18 protein is characterized by two functional domains: the isopeptidase activity domain and the IFNAR2-binding domain. The isopeptidase domain of this protease is composed of three catalytic domains, the palm, the thumb, and the finger; and the catalytic core consists of three amino acid residues (cysteine, aspartate, and histidine) localized between the thumb and palm domains (23). Thus, the catalytic activity of USP18 is due to its structure that contains cysteine and histidine boxes crucial for de-ISGylation (11, 24, 25). In humans, two isoforms of USP18 have been described: the USP18 isoform and the *N*-terminal truncated USP18-sf isoform, which differ in their amino-terminal regions. Although both isoforms are present in the cytoplasm, the USP18-sf isoform has also been observed in the nucleus (22, 26). Hence, USP18 is mainly located in the cytoplasm, while USP18-sf is evenly distributed in the cytoplasm and the nucleus. Although both proteins maintain their enzymatic and IFNAR2 binding abilities, the USP18-sf isoform is the main de-lSGylation enzyme for nuclear proteins. In general, USP18 protein has been found in many tissues, including the thymus, liver, spleen, lungs, bone marrow, adipose tissues (22), and various cancerous tissues (27).

USP18 has been reported to be involved in several biological responses to bacterial and viral infections (28–31), cell signaling pathways (16, 32–35), autoimmunity (36), malignant neoplasms (37, 38), neurological disorders and stress (39), and cardiac remodeling (40). Some studies have observed deregulated expression of *USP18* in some cancer types (24, 27, 37), including in bladder cancer (41), breast cancer (42), melanoma (38), and lung cancer (43). Interestingly, USP18 is also involved in the development of neurological disorders. Some studies involving mice with deficient USP18 have reported many neurological problems, including tremors, seizures, and hydrocephalus with a reduced lifespan (44, 45). Further studies are required to elucidate the molecular pathways associated with the role of USP18 in different physiological and pathological conditions. However, the role of USP18 as an IFN-signaling regulator and a de-ISGylase seems to be central in cancer, particularly in malignant mammary tumors. Here, we discuss the modulation and functions of the USP18 in breast cancer.

IFN-I SIGNALING PATHWAY AND ITS REGULATION BY USP18

As previously mentioned, many signaling pathways are involved in the regulation of USP18. It has been found that *Usp18* is rapidly and strongly upregulated after viral infection, LPS, TNF-α, genotoxic stress, and mainly by type I IFNs (IFN-I) (22). It has been reported that pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs). A classic example of this signaling is the detection of viral RNA by retinoic acid-inducible gene 1 (RIG-1) and toll-like receptor 3 (TLR3) (46). After recognizing these molecules, TLRs dimerize and activate the production of IFNs (19, 47). IFN-I is an antiviral cytokine group that includes several subtypes, among them IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω. The receptors for these IFNs are composed of two subunits, namely IFNAR1 and IFNAR2, which are associated with tyrosine kinase 2 (TYK2) and Janus-activated kinase 1 (JAK1), respectively. Once IFN-I binds to its receptor, dimerization and activation of associated kinases occur, promoting the phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2. Hence, phosphorylated STAT1 (p-STAT1) and p-STAT2 assemble into a heterodimer, translocate to the nucleus, and form a trimer with interferon regulatory factor 9 (IRF9), leading to the generation of the interferon-stimulated gene factor 3 (ISGF3) complex. The ISGF3 complex then binds to the IFN promoter to induce transcription (48). IFNs can modulate gene expression in a cell type-dependent manner. One of the IFN-stimulated genes (ISGs) is *USP18* (16). *USP18* expression seems to be important for the antiviral response since it has been observed that the absence of USP18 confers resistance to various viruses in mice (29, 49).

IFN-I treatment can induce apoptosis associated with prolonged phosphorylation of STAT (50, 51). However, this and other actions modulated by IFN-I can be inhibited by USP18. Hence, IFN-I signaling via the JAK/STAT pathway is negatively regulated through the binding of USP18 to the IFNAR2 subunit, blocking the binding of JAK1 to the IFN-I receptor complex (25, 49). This competitive inhibition occurs because the C-terminal portion of USP18 binds to the Box1-Box2 cytoplasmic region from the IFNAR2 intracellular domain, inhibiting the binding of JAK1 kinase (25). Additionally, the C and N-terminal regions of USP18 bind to the intermediate part of STAT2, whose function is to bind DNA. Furthermore, the removal of STAT2 can reduce the interaction between USP18 and IFNAR2 (35).

Several studies have demonstrated the importance of USP18 in IFN-I signaling. For example, USP18 knockout (KO) mice displayed brain damage and decreased life expectancy, with inflammation and overactivation of the microglia, characterized by STAT1/2 hyperphosphorylation and the overexpression of ISGs. This USP18 KO phenotype is rescued by IFNAR1 depletion, demonstrating that USP18 is a critical regulator of the IFN-I signaling pathway (24, 52). The phenotype of the USP18 KO animal model is in accordance with the observations in humans reported by Meuwissen (52), where a *USP18* mutation was found to result in loss of function and absence of the protein. People with this mutation develop Pseudo-TORCH syndrome, characterized by microcephaly and calcifications in the brain (52).

Ruxolitinib has been used to inhibit kinases in patients with USP18 deficiency (53). Moreover, in the pancreas, USP18 inhibition leads to inflammation, IFN-dependent B-cell apoptosis, and an increase in JAK/STAT signaling (54). Therefore, USP18 has been proposed as a target to enhance the therapeutic effect of IFN against viral infections or modulate IFN-I signaling in cancer (55–57). It is important to note that the deficiency of USP18 also affects other signaling pathways, leading to increased NF-κB and TNF-α signaling, and high levels of conjugated ISG15. Thus, these studies suggest that USP18 can also regulate itself through a negative feedback mechanism, either by inhibiting the binding of JAK1 to the IFNAR2 subunit, or by inhibiting the ubiquitination of the TAK1-TAB complex of TNFR1, another inductor of USP18 expression (22).

USP18 AS A REGULATOR OF PROTEIN ISGYLATION

ISGylation is a post-translational modification that covalently adds ISG15 to target proteins. ISG15 is a 15 kDa ubiquitin-like protein (58) generated by removing eight amino acid residues at the C–terminal, and the N–terminal methionine from a 165 amino acid long precursor protein of ISG15. The mature form of ISG15 (15 kDa) consists of two Ub–like domains that are connected via a "hinge" sequence. The C-terminus of ISG15 displays the motif LRLRGG, which is required to covalently bind to its target proteins via the ISGylation (59, 60). The ISGylation system is similar to ubiquitination and ubiquitin-like protein systems, involving three enzymes, the E1 activating enzyme, the E2 conjugating enzyme, and the E3 ligase enzyme, which is the last enzyme responsible for covalently and specifically binding ISG15 to the target protein (Figure 2). In particular, the ISGylation system consists of E1, E2, and only three E3 ligases, namely HERC5, HHARI, and EFP/TRIM25 (61–64). However, few ISGylation targets have been identified so far, in comparison with those of other posttranslational modifications. ISGylation alters protein–protein interactions, modifications, as well as cellular activities (59). An important finding is that USP18 de-ISGylates proteins and is therefore considered a critical means of regulating ISGylation. Thus, protein de-ISGylation mediated by USP18 involves releasing ISG15 in its free, non-conjugated state (12, 13).

USP18 without protease activity (USP18 C61A, inactive catalytic site) leads to an increase in ISGylation but does not affect IFN production. Furthermore, USP18 knock-in mice without enzymatic activity did not show IFN hypersensitivity, which indicated that the enzymatic activity is not related to other USP18 functions (65). Moreover, some studies have shown that USP18 deficient mice display ISGylation enhancement (25, 45, 60, 66). Therefore, USP18 activity appears to be a mechanism to reduce protein ISGylation, promoting the accumulation of free ISG15 (65, 67). Also, it has been reported that surviving USP18-deficient mice have high levels of conjugated ISG15, and display hydrocephalus and neurodegeneration (45). In addition, the deletion of USP18 has been associated with an increase in ISGylation at the feto-maternal interface (66).

As few ISGylation targets have been identified so far, the actions of USP18 on specific substrates have been completely defined. Interestingly, one study

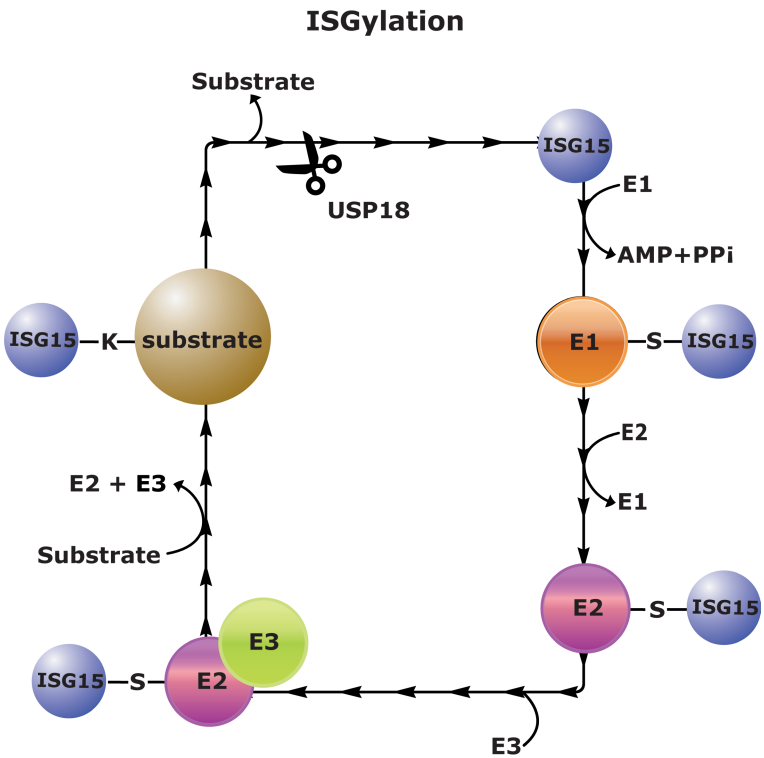

Figure 2. USP18 is a regulator of ISGylation. The protein ISGylation is mediated by the E1-activating (UBE1L), E2-conjugating (UBCH8), and E3-ligase (HERC5, TRIM25, HHARI) for ISG15. In this system, USP18 removes ISG15 from ISGylated protein.

identified a novel ISGylation target, protein phosphatase, and tensin homolog (PTEN). PTEN is an important tumor suppressor protein that, when ISGylated, becomes less stable. The authors observed that USP18 overexpression promoted PTEN de-ISGylation, leading to an increase in its stability, whereas the reduction of USP18 decreased PTEN stability. Thus, in lung cancer, a positive correlation between USP18 and PTEN protein levels has been observed (68).

ISG15, USP18 and IFN-I signaling pathways

IFN-I signaling pathways can promote antiviral responses, have immunomodulatory effects, and inhibit cell proliferation. ISG15 has been found to be induced by IFN-I and IFN-II (69, 70), associated with different cellular processes. For example, it has been reported that ISG15 KO mice are more susceptible to infection by influenza viruses A and B, and by the herpes simplex virus type 1 (46, 71, 72), while in the same mice with ISG15 deficiency, infection by lymphocytic choriomeningitis virus (LCMV) or vesicular stomatitis virus (VSV) does not appear to be different from wild-type mice (73). Thus, ISG15 can exhibit antiviral activity against some viruses, but not against others.

In addition, the functions of ISG15 depend on its status as a free or conjugated protein. Free ISG15 acts as a cytokine that activates NK cells and CD8+ T cells, promotes dendritic cell maturation, and participates in innate and adaptive immune responses (46). Furthermore, free ISG15 is also present intracellularly, regulating protein-protein interactions implicated in signaling pathways. Both free ISG15 and ISGylation have been found to be associated not only with antiviral responses, but also with the pathology of several cancer types, including breast cancer (59).

As USP18 is an inhibitor of IFN-I signaling, and a de-ISGylase, the interplay between these two functions may be deregulated in some pathologies, including cancer. It has been reported that intracellular free ISG15 can stabilize USP18 levels in humans. USP18 stability is conferred by the interaction between ISG15 and USP18, which blocks its SKP2-induced degradation (34). Specifically, free ISG15 promotes the disassembly of SKP2 from USP18 to avoid degradation of USP18 (74). Thus, intracellular-free ISG15 may contribute to the inhibition of IFN-I signaling pathways by USP18.

USP18 AS A REGULATOR OF ISGYLATION OF BREAST CANCER PROTEOME

Many studies have recently emerged on ISGylation in breast cancer. For instance, in MDA-MB-231 breast cancer cells, the use of interfering RNA (siRNA) to reduce the expression of *ISG15* leads to a decrease in the proliferation, migration, and epithelial-mesenchymal transition (EMT). A similar result is observed by the use of siRNA for *UBCH8,* an E2-conjugating enzyme for ISG15 (75). In contrast, high levels of *ISG15* or *UBCH8* expression are correlated with enhancing the tumorigenic characteristics of breast cancer cells. These actions of ISG15 have been related to protein ISGylation and its potential to interfere with protein ubiquitination in breast cancer cells (75, 76). Nevertheless, the same research group also demonstrated that *in vivo* ISG15 can have antitumor effects based on xenotransplantation of ISG15 knockdown ZR-75-1 or MDA-MB-231 breast cancer cells in athymic (nude) mice, showing an increased tumor development and decreased of NK cells recruitment. Interestingly, xenotransplanted MDA-MB-231 cells in athymic mice were reduced by a subcutaneous injection of recombinant purified ISG15, and high tumor NK cell infiltration and expression of major histocompatibility complex (MHC II) were observed (77).

The reason behind the seemingly opposite effects of ISG15 on the tumorigenic characteristics *in vitro* and *in vivo*, observed using siRNA, remains unclear. However, the intracellular actions of free ISG15 and conjugated ISG15 may be modulated by the interactions between cells and the extracellular matrix, which differ between *in vitro* and *in vivo* studies. Hence, the activity of extracellular free ISG15 is cytokine-like and has been shown to inhibit tumor growth in breast cancer xenotransplants in athymic mice (77), suggesting that USP18 may play a central role in the development of malignant mammary tumors. As USP18 facilitates the accumulation of free ISG15, de-ISGylation may transcend the progression of breast cancer (Figure 3).

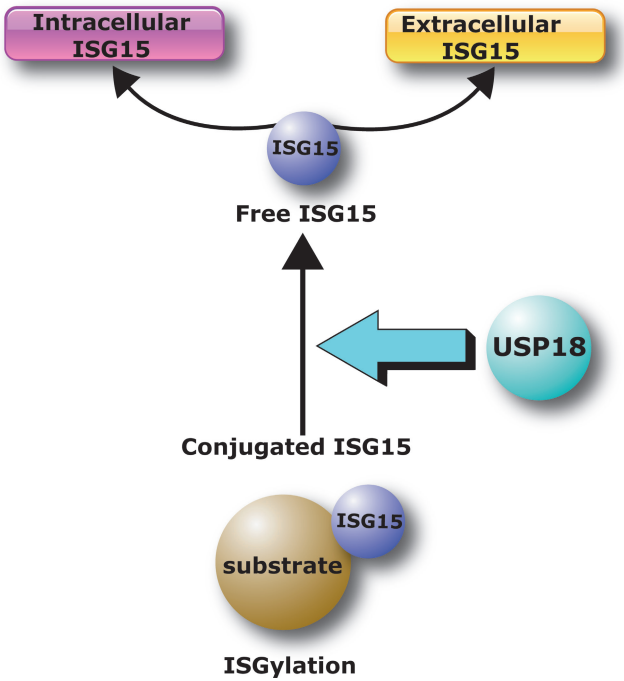

Figure 3. There is an interplay between USP18 and free ISG15. The de-ISGylation activity from USP18 affects the levels of free ISG15 since USP18 removes ISG15 from ISGylated protein. Free ISG15 can have extracellular functions as a cytokine-like protein and intracellularly by modulating some protein interactions.

USP18 AND BREAST CANCER MICROENVIRONMENT

To investigate the effect of USP18 on breast cancer, Burkart et al. (78) performed a study in which they used a polyomavirus middle T (PyVmT) mouse model for breast cancer and included two groups: PyVmT/Usp18 KO and PyVmT/Usp18 wild type (WT). At 13 weeks of age, PyVmT/USP18 WT mice were sacrificed due to the presence of multiple large tumors, while PyVmT/USP18 KO mice showed a clear reduction in the size and number of visible tumors. This result may be explained by the fact that PyVmT/USP18 KO tumors had a significant decrease in CD31 positive cells, indicating an angiostatic effect of USP18 deficiency. Furthermore, a significant increase in the number of activated $CD4^+$ T cells was observed in the tumors of PyVmT/Usp18 KO mice, compared to PyVmT/Usp18 WT mice. Interestingly, it was observed that PyVmT/USP18 KO mammary epithelial cells (MECs) secrete elevated levels of CXCL10, creating a Th1/M1-polarized cytokine tumor environment, thereby exhibiting a Th1-like cytokine profile, characterized by increased IFN-γ and decreased IL-4 and IL-13 levels. Furthermore, the authors reported that IFN-I promotes CXCL10 upregulation in MECs. As USP18 is an inhibitor of IFN-I signaling, the absence of USP18 facilitates an IFN-I-dependent CXCL10 upregulation. Knockdown of IL-28R1 in USP18-deficient MECs dramatically enhanced tumor growth. This study suggests that USP18 may

be a regulator of the tumor microenvironment and is therefore a promising candidate for breast cancer immunotherapy (78).

In addition, cancer-associated fibroblasts (CAFs) play critical roles in tumorigenesis and chemoresistance. Recently, it has been reported that $CD10^+$ $GPR77^+$ CAFs favor breast cancer stemness by providing a favorable microenvironment for cancer stem cells. To identify critical co-expression modules and calculate the module-trait relationship (MTR), Xu et al. (79) applied a weighted gene co-expression network analysis (WGCNA) of transcriptomic data, from CAFs collected from chemoresistant or chemosensitive breast cancer patients before neo-adjuvant chemotherapy (NAC). As a result of this analysis, 12 modules were determined; although only one of them was associated with CAF-related chemoresistance, and was related to "inflammatory response", "interferon-gamma-mediated signaling", and "NIK/NFκB signaling" pathways, and included the enriched hub genes of *CXCL8*, *CXCL10*, *CXCL11*, *PLSCR1*, *RIPK2* and *USP18*. Moreover, the researchers validated these six genes by RT-qPCR and reported that all of them showed a higher expression in the breast cancer CAFs of chemoresistant patients than those of the chemosensitive ones. Finally, the overall survival data demonstrated that the prognosis of breast cancer patients was poorer when these hub genes were highly expressed (79). These data indicate that *USP18* is part of a signature of genes implicated in breast cancer progression.

EGF/EGFR SIGNALING AND USP18 IN BREAST CANCER

The high complexity of oncogenic pathways has led to the pursuit of strategies capable of inhibiting cancer cell proliferation, and among the studied factors is the epidermal growth factor receptor (EGFR) (80). EGFR is a tyrosine kinase that triggers an intracellular signal transduction pathway, that promotes cellular proliferation; therefore, targeting EGFR tyrosine kinase activity, either with inhibitors or with antibodies, has been proposed as a strategy for tumor treatment (81). Interestingly, USP18 was identified as a candidate to regulate EGFR. This study observed that the reduction of USP18 expression by a siRNA leads to - miR7 upregulation, affecting the translation of miR7-target mRNAs, one of them, EGFR. Thus, an increase in the miR7 levels due to the depletion of USP18 generates a reduction in the EGFR protein levels. These events lead to apoptosis, making EGFR modulation a potential new therapy for tumor treatment (81).

In 2018, Tan et al. (42) performed bioinformatics analysis and *in vivo* experiments to explain the molecular mechanisms that underlie the effects of USP18 on the progression of breast cancer. They reported that low methylation in the promoter region of *USP18* leads to its upregulation in breast cancer. Furthermore, *USP18* mRNA levels were positively correlated with the epithelial cell proliferation gene set, suggesting that USP18 promotes breast cancer cell growth, and was negatively associated with the cell cycle arrest gene set and apoptosis gene set in patients with breast cancer. These results demonstrate that USP18 may participate in breast cancer development by promoting cell cycle progression and inhibiting apoptosis. Moreover, *USP18* expression was shown to increase *EGFR* expression and activate the AKT/SKP2 pathway. Breast cancer patients with high *USP18* and *SKP2* expression had worse survival rates (42). Remarkably, *USP18* was

significantly upregulated in patients with HER2+ breast cancer, and increased expression of *USP18* and *SKP2* was associated with the worst prognosis (42). These results suggest that *USP18* and *SKP2* may be useful as markers in breast cancer development.

USP18 AND THE TREATMENT FOR BREAST CANCER

Tamoxifen (TAM) is a SERM drug used for breast cancer treatment, as it interferes with the expression of estrogen-regulated genes in breast cancer cells. Recently, Fang et al. (82) conducted a study in which they showed that after a 72 h-treatment with TAM, MCF-7 breast cancer cells showed a decreased cell growth rate in a dose-dependent manner. Transcriptomic analysis revealed that TAM-treated MCF-7 cells, when compared to the control culture cells, had 332 upregulated genes, including the genes for *STAT1, STAT2, EIF2AK2, TGM2, DDX58, PARP9, SASH1, RBL2,* and *USP18*; and 320 downregulated genes, including the genes for *CCDN1, S100A9, S100A8, ANXA1*, and *PGR*. Thus, these results suggest that *USP18* is an upregulated gene by TAM. Furthermore, in human breast tumor tissues, mRNA levels of some genes, among them *USP18*, were significantly higher in ERα+ breast cancer tissues than in ERα- ones. Moreover, the mRNA levels of USP18 were significantly higher in ERα+ tumor tissues than in their corresponding tumor-adjacent tissues. Taking these results into account, it seems that the effects of TAM on breast cancer cells rely on the regulation it exerts on the genes mentioned above, one of them being *USP18*, which seems to be also involved estrogen signaling pathways (82).

FUTURE CHALLENGES IN ELUCIADITING THE ROLE OF USP18 IN BREAST CANCER

Deubiquitinases remove ubiquitination and ISGylation modifications, which are important posttranslational modifications influencing protein function (12). Prior studies have revealed that USP18 acts by deISGylating substrate proteins; thus, USP18-deficient mice showed increased levels of intracellular ISG15 conjugates, and *in vitro* analysis showed that USP18 removes ISG15 from ISGylated proteins, but it has no effect on modified proteins by other ubiquitin-like proteins (12, 65). This finding suggests that USP18 is an ISG15-specific protease. However, there are particular challenges to be addressed in the future. The first challenge is the need for additional studies to identify ISGylation targets and elucidate how ISGylation affects protein functions in several biological processes, including breast cancer progression (27). Consequently, it will be possible to understand the implications of de-ISGylation of these proteins by USP18 in mammary tumorigenesis.

A second challenge is the demonstration and application of USP18 as a target of antineoplastic strategies, based on the observation that USP18 mRNA and protein levels are high in most types of cancer, including breast cancer, and that inhibition of USP18 expression is associated with favorable cancer-specific survival (41). Individuals expressing low levels of USP18 had a higher survival percentage than those that expressed high levels, suggesting that upregulation of USP18 is a significant risk factor for cancer-related death (10).

Thus, specific inhibitors of USP18 may be used as novel breast cancer therapeutics. Furthermore, with the crystal structure of murine USP18 being solved in isolation and in combination with ISG15, it will be easier to develop the next generation of USP18 inhibitors and search for antineoplastic effects (27). Determining the effect of USP18 on the ISGylome of human breast cancer cells represents another challenge. A study demonstrated that ISGylation is not involved in protein degradation; instead, ISGylation is involved in protein stabilization, perhaps through co-translational modification in chronic myeloid leukemia cells. Interestingly, they showed that USP18 deficiency sensitizes cancer cells to radiotherapy in a dose-dependent manner, probably by interfering with IFN signaling, and that USP18-deficient cells are more antigenic, which enhances the activation of cytotoxic T lymphocytes (83). Moreover, USP18 has also been associated with inflammation and resistance to chemotherapy, and USP18 inhibition has been suggested to improve the response during chemotherapy (10, 51). Thereby, the multifunctionality of USP18 may also be critical in breast cancer progression and the response to therapeutic strategies (Figure 4).

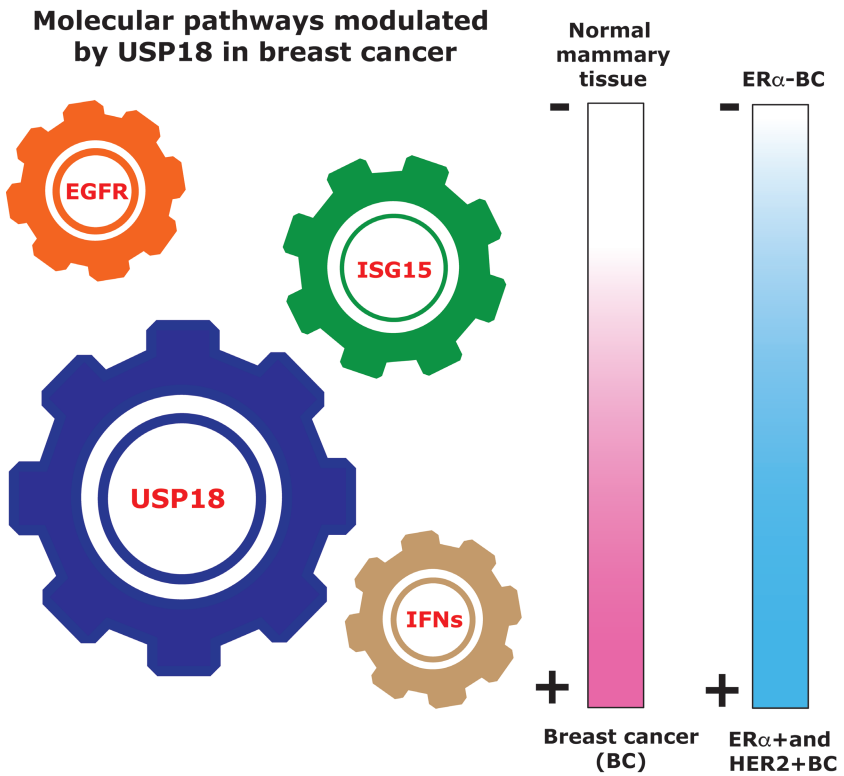

Figure 4. USP18 in breast cancer. USP18 modulates molecular pathways activated by IFNs, ISG15/ISGylation, and EGFR in breast cancer cells. The USP18 mRNA/protein levels are increased in breast cancer tissue, mainly in the ERα+ and HER2-overexpressing subtypes.

Finally, considering the functional complexity of ISG15 in the context of breast cancer, another challenge will be to decode the activity of USP18 in ISG15-dependent and independent pathways involved in the progression of malignant mammary tumors. Likewise, it is required to integrate the role of USP18 in ISG15-dependent and independent pathways in breast cancer.

CONCLUSION

USP18 is a multifunctional protein, and additional studies are required to fully elucidate its role in breast cancer. Moreover, its function as a negative regulator of IFN-I pathway, and as an enzyme that modulates ISGylation, leads to the hypothesis that USP18 may be centrally involved in the development and progression of breast cancer. Consequently, USP18 may be useful as a biomarker, as well as a novel molecular target for new therapeutic strategies in this pathology.

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