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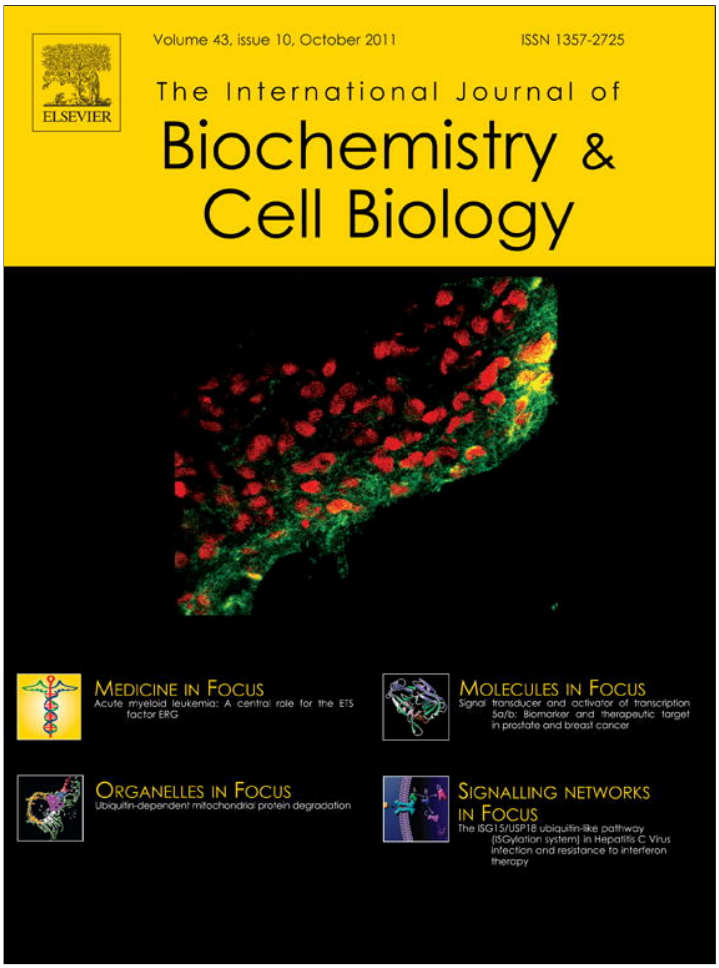

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Signalling networks in focus

The ISG15/USP18 ubiquitin-like pathway (ISGylation system) in Hepatitis C Virus infection and resistance to interferon therapy

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ABSTRACT

The ISG15/USP18 pathway modulates cellular functions and is important for the host innate immune response to chronic viral infections such as Hepatitis C Virus (HCV). Interferon stimulated gene 15 (ISG15) was the first ubiquitin-like protein modifier identified. As in ubiquitination, ISG15 conjugates to target proteins (ISGylation) through the sequential enzymatic action of activating E1, conjugating E2, and ligating E3 enzymes. ISGylation modulates signal transduction pathways and host anti-viral response. The ISGylation process is reversible through the action of an ISG15 protease, USP18. Ubiquitin-like specific protease 18 (USP18) has functions that are both ISG15-dependent and ISG15-independent; the importance of the ISG15/USP18 pathway to chronic HCV infection is illustrated by the consistent finding of increased levels of ISG15 and USP18 in the liver tissue of patients who do not respond to interferon-based treatments. Mechanistically, HCV seems to exploit the ISG15/USP18 pathway to promote viral replication and evade innate anti-viral immune responses.

Signaling network facts

- Interferon stimulated gene 15 (ISG15) was the first ubiquitin-like protein modifier identified, containing two ubiquitin-like domains at both termini which share 32–33% homology with ubiquitin.
- ISG15 exists in free and conjugated forms in vivo. Conjugation of ISG15 to its target proteins (ISGylation) is induced in various viral/bacterial infections and in response to interferon treatment.
- ISGylation requires the sequential action of an E1 activating enzyme (Ube1L), an E2 conjugating enzyme (UbcH8) and an E3 ligase (EFP, HHARI and Herc5), all of which are interferon inducible.
- ISGylation has been involved in various functions, though its role in viral infections has been most comprehensively studied.
- Ubiquitin-like Specific Protease 18 (USP18) is a cysteine-protease that cleaves ISG15 from its conjugated targets; USP18 has both protease-dependent and -independent functions.
- The ISG15/USP18 pathway is altered in HCV-infected liver tissue and plays an important role in modulating HCV infection and resistance to interferon therapy.

1. Introduction

Interferon stimulated gene 15 (ISG15) was first described in 1979 as a 15 kDa protein (Farrell et al., 1979). The first human sequence was cloned in 1986 and was labeled as ubiquitin cross-reactive protein (UCRP) due to cross-reaction with anti-ubiquitin antibodies. This cross-reactivity is likely secondary to structural similarities between the ubiquitin-like domain of ISG15 and ubiquitin (Fig. 1
).

The full-length human ISG15 gene encodes a 165aa protein as a precursor with a 8aa overhang at the c-terminus. This is cleaved to expose the essential conjugating sequence LRLRGG (Knight et al., 1988). Like ubiquitin, ISG15 conjugates to lysine residues of target proteins via its C-terminal Glycine-Glycine motif. Conjugation (ISGylation) occurs through the sequential enzymatic reaction of an E1 activating enzyme (Ube1L), an E2 conjugating enzyme (UbcH8), and an E3 ligase (EFP, HHARI and Herc5). In cells treated with type I interferon (IFN) or exposed to pathogens, both free form ISG15 and ISGylation are increased.

2. Biological functions of ISG15 and its conjugation

ISG15 is one of the most abundant ISGs induced by type I IFNs or by viral/bacterial infections (Fig. 1). Viral infection activates the host innate immune system to induce type I interferon through RIG-I and TLR3 pathways. After IFNβ is expressed and binds to its

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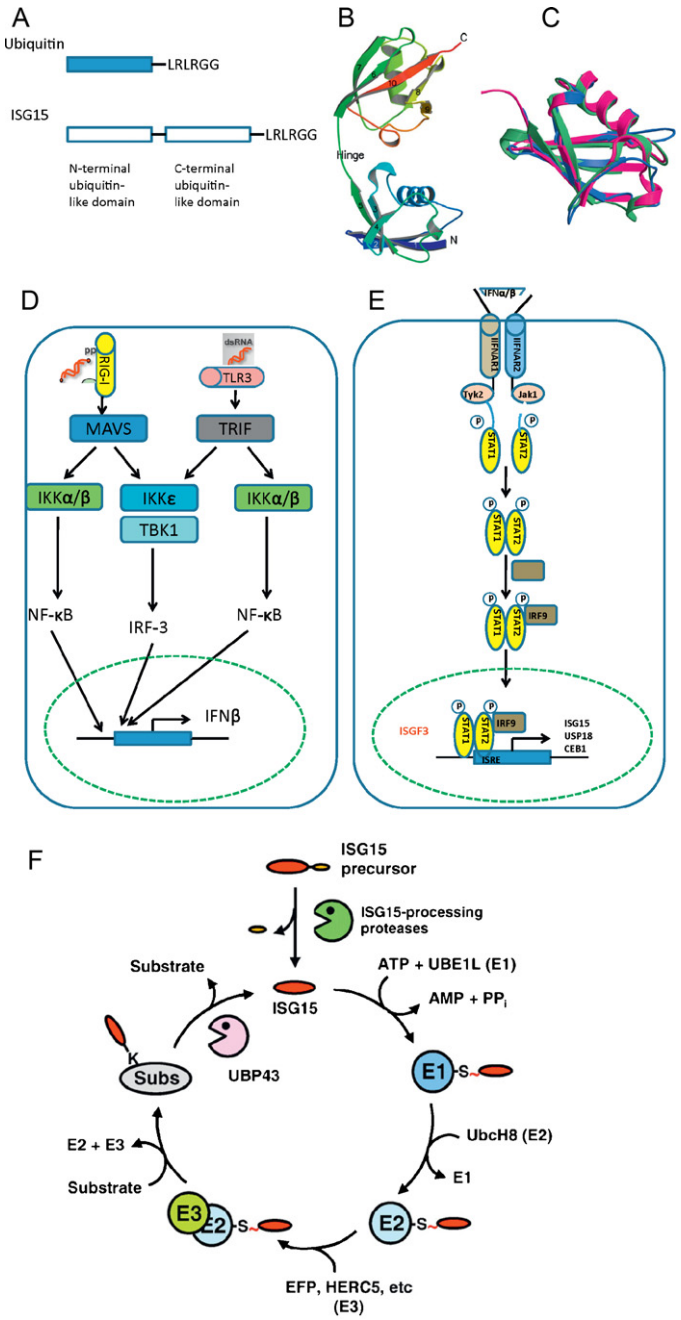

Fig. 1. ISG15 structure, type I interferon production, Jak/STAT signaling and ISG15 conjugation pathway. Ubiquitin and ISG15 domain structure (A). Note that ISG15 contains 2 ubiquitin-like domains with the same C-terminal conjugation motif Leu Arg Leu Arg Gly Gly to conjugate to the Lysine residues of the target proteins. Ribbon diagram of ISG15 showing two separate domains, with color ramped from blue (N terminus, N) to red (C terminus, C) through green (Hinge) (B). The two ubiquitin-like domains (each in β-grasp fold) are oriented differently and connected by a hinge. Overlay of ribbon diagrams for ubiquitin (pink) with the amino- (blue) and C-terminal (green) domains of ISG15 to emphasize the marked similarities in their respective β-grasp folds (**C**). Figure is adapted from Narasimhan J et al. J. Biol. Chem. 2005;280:27356–27365. Type 1 interferon production upon virus infection (D). Host cells sense viral infection with two independent sensor molecules (RIG-I and TLR3). RIG-I (DDX58) is a DExD box helicase expressed ubiquitously within the cytoplasm of most cell types. It senses short non-self double-stranded RNAs (dsRNAs) with free 5′-triphosphates and translocates to the mitochondria surface to interact with adaptor protein MAVS (mitochondrial antiviral signaling protein; also known as IPS-1, VISA, and Cardif) through shared caspase recruitment domains (CARDs) in RIG-I and MAVS. TLR3 senses dsRNA formed during the replication of positive-strand RNA viruses as well as some DNA viruses. This recognization drives TLR3 dimerization that results in recruitment of an adaptor protein, TRIF (TIR domain-containing adaptor-inducing IFN-β; also known as TICAM-1), that interacts with TLR3 through homotypic TIR domains. Association of the adaptor proteins with RIG-I and TLR3 converges to activate down stream transcription factors IRF-3 and NFκB through IKKε/TBK1 and IKKα/β, respectively. The activated IRF-3 and NFκB are translocated into the nucleus to stimulate IFNβ production. Jak/STAT signaling pathway (E). Type I IFNs (IFNα and IFNβ) bind to the same cognate surface receptor IFNAR to induce STAT1 and STAT2 phosphorylation and dimerization through Jak 1 and Tyk2. Together with IRF9, STAT1/STAT2/IFR9 form a transcription factor complex called ISGF3 that is translocated into the nucleus to bind to the interferon stimulate response element (ISRE) in the promoter region of the ISGs, resulting in ISG15, USP18, CEB1 and other ISGs production. ISG15 conjugation pathway (F). ISG15 conjugates to its target proteins through E1 activating enzyme (Ube1L), E2 conjugating enzyme (UBcH8), and one of the E3 ligases (EFP, HHARI and Herc5). USP18 (also called UBP43), a cysteine protease, specifically cleaves ISG15 from its targets. Figure is adapted from Jeon YJ et al. BBA 2010; 1802:485–496.

cognate type 1 IFN receptors, IFNα and ISGs (including ISG15) are upregulated through the Jak/STAT pathway.

ISG15 exists in either a free or conjugated form. When secreted, free ISG15 can function as a cytokine that modulates immune responses. For example, free ISG15 can activate NK and cytotoxic T-cells, stimulate IFN-γ production, and induce dendritic cell maturation and neutrophil recruitment (D'Cunha et al., 1996). By contrast, intracellular ISGylation can modulate protein functions either directly or by competing for or altering ubiquitination. ISGylation of filamin B, a scaffold molecule that links activated Rac1 and JNK, inhibits type I IFN-induced JNK signaling pathway (Jeon et al., 2008). ISGylation of pp2cβ, a critical phosphatase that suppresses NF-κB signaling, activates NF-κB-dependent pathways (Takeuchi et al., 2006). ISGylation can also alter ubiquitination. This can occur by competition for shared conjugation sites: ISGylation competes with the ubiquitination of viral proteins such as the HIV Gag protein, and of host proteins such as interferon regulatory factor 3 (IRF3). In both cases inhibiting ubiquitination has functional consequences (respectively, inhibiting HIV release, and protection from degradation). ISGylation can also directly target proteins involved in ubiquitination. ISGylation of UbcH13, a ubiquitin-conjugating E2 enzyme, inhibits the generation of Lys63-linked poly-ubiquitin chains that are conjugated to a variety of signaling molecules (VanDemark et al., 2001). The E2 UbcH8 and UbcH6 enzymes are also targets for ISGylation (Takeuchi et al., 2005), and their ISGylation may inhibit their ubiquitin-conjugating activity.

Its effects on diverse cell signaling pathways imply that ISG15 and ISGylation play roles in many cellular functions. These include tumorigenesis (Yoshida et al., 1996; Kiessling et al., 2009; Desai et al., 2006), but the role of the ISG15 pathway in the host innate immune response has stimulated much research. The strong induction of ISG15 and of ISGylation by IFN and viral infection suggests an important role in host antiviral defenses. ISG15 has been shown to conjugate to over 160 host proteins (identified by mass spectrometry). Some of these target proteins are downstream effector ISGs of interferon signaling: PKR, MxA, Hup56, and RIG-I (Zhao et al., 2005), while others are involved in the regulation of type I IFN signaling, such as PLCγ1, JAK1, ERK1, and STAT1 (Malakhov et al., 2003). Whatever the global effect of the ISGylation of cellular proteins, the ultimate role of ISG15/ISGylation is clearly virus-specific. For example, ISG15 knock-out mice are more susceptible to infection with Sindbis virus, influenza A and B viruses, herpes simplex virus type 1 (HSV-1) and murine gammaherpesvirus 68 (Lenschow et al., 2007). ISGylation is likely important for the protective role of ISG15 against sindbis virus and Influenza B: knocking out the E1 UBE1L, or mutating the Arg151 residue of ISG15 (critical for its interaction with UBE1L), renders mice more susceptible to these infections (Lai et al., 2009). On the other hand, mice lacking ISG15 or UBE1L have no apparent defect in defense against vesicular stomatitis virus (VSV) or lymphocytic choriomeningitis virus (LCMV) (Osiak et al., 2005).

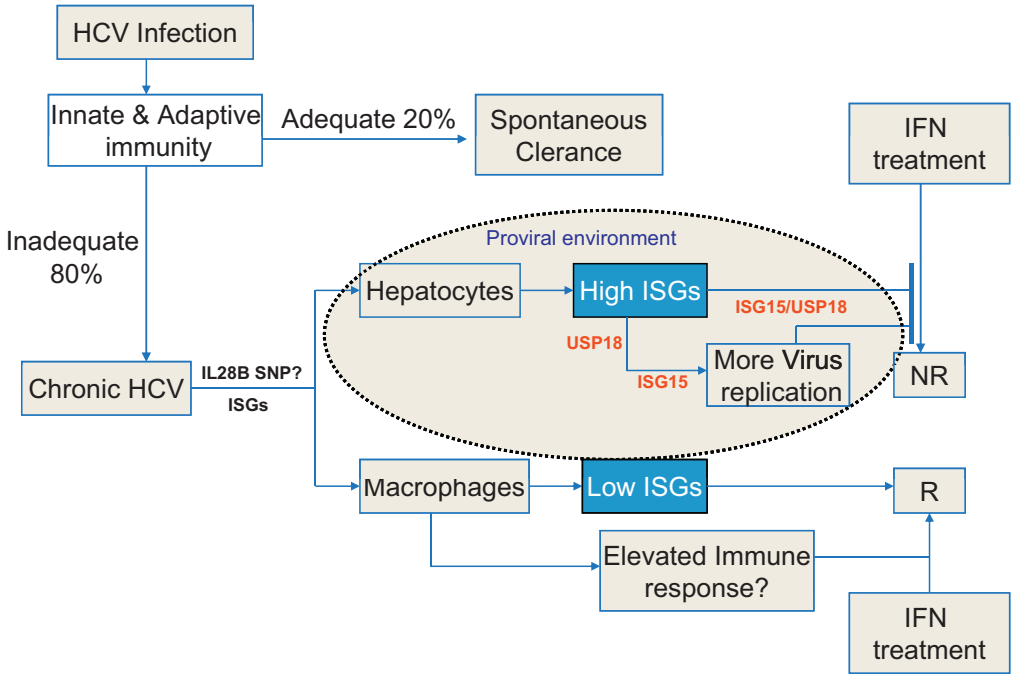

Fig. 2. ISG15/USP18 ubiquitin-like pathway in HCV infection and resistance to treatment. HCV infection induces host innate and adaptive immune responses. 20% of those infected patients whose immune response is adequate will spontaneously clear the virus while majority (80%) will develop into chronic infections. In these HCV chronically infected livers, IFN and ISGs induced in different cell types (probably due to the restricted type III IFNλ receptor expression and IFNλ3-IL28B polymorphism). If the ISG induction is mainly focused in hepatocytes, the baseline expression levels of these ISGs are high due to the fact that hepatocytes are the most abundant cell types in the liver tissue. Increased expression of ISG15 and USP18 will not only promote HCV replication but also blunt IFN anti-HCV activity. As a result, the IFN treatment is not effective and patients are not responding. On the other hand, if the ISGs are expressed mainly in the liver macrophages, the baseline expression levels of these ISGs are low due to limited number of macrophages present in the liver. Furthermore, expression of ISGs in the macrophage may indicate that macrophages are in the active state that play an important role in inducing adequate immune response to render patients responsive to IFN therapy.

3. Role of USP18

ISG15 can be stripped from its target proteins by the ISG15-specific cysteine protease Ubiquitin-like specific Protease 18 (USP18). Like ISG15/ISGylation, USP18 plays an important role in the host innate immune response. This role is mediated both via its ISG15 protease action and in protease-independent fashions. USP18 is induced by IFN, LPS, and viral infections, and can modulate type I IFN responses (Malakhova et al., 2006). Deletion of the USP18 gene in mice leads to IFN hypersensitivity (Ritchie et al., 2004). USP18−/− cells treated with IFN have increased and prolonged STAT1 phosphorylation, increased ISG induction, and increased levels of apoptosis (Ritchie et al., 2004). USP18−/− mice also have a greater resistance to the cytopathic effects of a number of viruses including LCMV, VSV, and sindbis virus (Ritchie et al., 2004). These actions are not mediated always through the ISG15 protease activity of USP18: if this were so, then the phenotype of the USP18−/− mice would be predicted to be reversed, or at least affected, by loss of either ISG15 or of its E1 activating enzyme Ube1L, but in fact there was no effect of either (Osiak et al., 2005; Kim et al., 2006). As a result, several have hypothesized that USP18 may influence immune function by interacting directly with proteins involved in immune regulation. Indeed murine USP18 binds to the IFN-receptor (IFNAR2) and blocks IFN signaling by disrupting IFNAR2-JAK binding (Malakhova et al., 2006). These data argue that USP18 can bind to and modulate the activity of cellular proteins independent of its ISG15 protease function.

4. Cascades and key molecules

As noted above, the ISG15 conjugation system consists of an E1 activation enzyme (Ube1L), an E2 conjugation enzyme (UbcH8), and several E3 ligases (EFP, HHARI and Herc5). The USP18 isopeptidase is the major ISG15-specific protease, and cleaves ISG15 from its conjugated targets.

4.1. ISG15-activating E1 enzyme Ube1L

Ube1L is the only activating enzyme for ISG15 conjugation system discovered to date. Ube1L-deficient mice cannot produce ISG15 conjugates upon stimulation, while ubiquitination is intact (Kim et al., 2006). The Ube1L gene, which encodes a 112-kDa protein with 45% identity with ubiquitin activating E1 enzyme, was cloned from a human pre-B cell library in 1993 and was mapped to chromosome 3q21. Like ISG15 and the other enzymes required for ISG15 conjugation, Ube1L expression is up-regulated by IFN treatment.

4.2. ISG15-conjugating E2 enzymes UbcH8 and UbcH6

The UbcH8 E2 ubiquitin conjugating enzyme was identified as an E2 enzyme for ISG15 in 2004 (Zhao et al., 2004). Consistent with it being an ISG, the UbcH8 promoter region contains an IFN stimulated response element (ISRE) site that can bind ISGF3. The UbcH8 gene was mapped to chromosome 11q12 and encodes a 16 kDa protein. UbcH6 is also up-regulated by type I IFN and forms lower levels of thioester intermediates with ISG15 than UBcH8, indicating that UBcH6 is a minor E2 for ISG15.

4.3. ISG15 E3 ligases EFP, HHARI and Herc5

Since some ubiquitin E2 conjugating enzymes also serve as ISG15 E2s, it is possible that some UbcH8-interacting ubiquitin E3 ligases can also function as ISG15 E3 ligases. Indeed, estrogen-responsive finger protein (EFP), a UbcH8- interacting protein, is an E3 ligase for ISG15 conjugation to 14-3-3σ (Zou and Zhang, 2006). Similarly, HHARI (human homolog of Drosophila ariadne)

also serves as an ISG15 E3 ligase for 4EHP (Okumura et al., 2007). Unlike EFP and HHARI, both having restricted substrate specificity, Herc5 (HECT domain and RCC1-like domain containing protein 5) has been identified as a third E3 ligase for ISG15 but without substrate specificity (Wong et al., 2006).

4.4. ISG15 deconjugating enzymes

USP18 is the major ISG15-speicifc protease that deconjugates ISG15 from its target proteins (Ritchie et al., 2002). Originally cloned in 1999, USP18 encodes a protein with calculated molecular weight of 43 kDa (thus, it is also termed UBP43). The human USP18 gene was mapped to chromosome 22q11.2 (Schwer et al., 2000). The overlapping functions of some E2 and E3 enzymes in the conjugation process of both ISG15 and ubiquitin implies the existence of promiscuous deubiquitinases (DUBs) that may serve as ISG15 proteases. Indeed, several DUBs, such as USP2, USP5, USP13, and USP14 can function as ISG15 precursor processing and/or deconjugating enzymes (Catic et al., 2007).

5. Role of the ISG15/USP18 pathway in a specific human infection: HCV

Our work and that of others demonstrates that ISG15/ISGylation and USP18 can play surprising and important roles in human viral infections. Treatment of HCV is based to a greater or lesser degree on the provision of exogenous IFNα, i.e., on upregulation of the host innate immune response. When we compared hepatic gene expression between patients who went on to respond to treatment and those who did not, we found a startling up-regulation of ISGs in the pre-treatment liver tissue of those who then did not respond to IFN-based treatment (Chen et al., 2005). Of the 18 genes that discriminated eventual treatment responders and nonresponders, three were part of the ISG15/USP18 pathway: ISG15, USP18, and CEB1 (Herc5). We initially hypothesized that ISG15/ISGylation was antiviral, and indeed silencing of USP18 led to increased ISGylation and potentiated the anti-viral effect of exogenous IFNa (Randall et al., 2006). However, the story is far more complicated. Increasing ISGylation independent of USP18 actually promotes efficient viral production (Chen et al., 2010a), and increasing USP18 promotes HCV production in a protease-independent fashion, while blunting the anti-viral effect of IFNa (Chen et al., 2008). The effect of ISG15 and USP18 on human liver infection is also very likely cell-specific. We have found that cell-specific induction of ISGs, including ISG15, is strongly associated with treatment responses (Chen et al., 2010b). Hepatocellular expression of ISG15 correlates with treatment non-response, macrophage expression of ISG15 with treatment response. Thus, the overall response of the host to HCV is a reflection not only of the interaction of the virus with ISG15 and USP18 in infected cells (hepatocytes), but of the effects of ISG15 and USP18 in hepatic immune cells (macrophages). Taken all these together, pre-activation of the ISG15/USP18 pathway in the hepatocytes leading to increased expression of ISG15 and ISGylation as well as baseline USP18 not only promotes HCV production but also inhibits IFN anti-HCV activity, rendering patients into persistent infections (Fig. 2).

6. Therapeutic implications

The diverse effects of the ISG15/USP18 pathway suggest that its modulation could play a therapeutic role in many disease states, particularly diseases that are influenced by the innate immune response. These might include cancers that are known to be sensitive to immune modulation (such as melanoma), or infections in which altering the host immune response alters the ability of the virus to replicate in or infect cells. As described in this review, individual viral infections will have different responses to manipulation of the ISG15/USP18 pathway. For HCV, in which ISGylation promotes viral production, Ube1L protease inhibitors aimed at decreasing ISGylation, or USP18 blockers that disrupt the pro-HCV effect of USP18 but leave other immune functions intact, might blunt the infection and increase the efficacy of other anti-viral agents. One advantage of modulating host immune functions such as the ISG15/USP18 pathway, rather than going after viral proteins alone, is that it should impede the ability of the virus to escape treatment by mutating. This is particularly true for HCV, a virus that is notoriously mutagenic and for which single agent-therapy has never succeeded.

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References

Catic A, Fiebiger E, Korbel GA, Blom D, Galardy PJ, Ploegh HL. Screen for ISG15-crossreactive deubiquitinases. PLoS One 2007;2:e679.

Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterology 2005;128(5):1437–44.

Chen L, Borozan I, Sun J, Guindi M, Fischer S, Feld J, et al. Cell-type specific gene expression signature in liver underlies response to interferon therapy in chronic hepatitis C infection. Gastroenterology 2010b;138(3), 1123-33.e1–3.

Chen L, Sun J, Meng L, Heathcote J, Edwards AM, McGilvray ID. ISG15, a ubiquitin-like interferon-stimulated gene, promotes hepatitis C virus production in vitro: implications for chronic infection and response to treatment. J Gen Virol 2010a;91(Pt. 2):382–8.

Chen L, Sun J, Meng XB, Edwards AM, Heathcote J, McGilvray ID. USP18 promotes HCV replication and blunt the antiviral effect of IFNα in a protease-independent fashion. Hepatology 2008;48(4):399A.

D'Cunha J, Knight Jr E, Haas AL, Truitt RL, Borden EC. Immunoregulatory properties of ISG15, an interferon-induced cytokine. Proc Natl Acad Sci USA 1996;93(1):211–5.

Desai SD, Haas AL, Wood LM, Tsai YC, Pestka S, Rubin EH, et al. Elevated expression of ISG15 in tumor cells interferes with the ubiquitin/26S proteasome pathway. Cancer Res 2006;66:921–8.

Farrell PJ, Broeze RJ, Lengyel P. Accumulation of an mRNA and protein in interferon-treated Ehrlich ascites tumour cells. Nature 1979;279(5713):523–5.

Jeon YJ, Choi JS, Lee JY, Yu KR, Ka SH, Cho Y, et al. Filamin B serves as a molecular scaffold for type I interferon-induced c-Jun NH2-terminal kinase signaling pathway. Mol Biol Cell 2008;19:5116–30.

Kiessling A, Hogrefe C, Erb S, Bobach C, Fuessel S, Wessjohann L, et al. Expression, regulation and function of the ISGylation system in prostate cancer. Oncogene 2009;8:2606–20.

Kim KI, Yan M, Malakhova O, Luo JK, Shen MF, Zou W, de la Torre JC, Zhang DE. Ube1L and protein ISGylation are not essential for alpha/beta interferon signaling. Mol Cell Biol 2006;26(2):472–9.

Knight Jr E, Fahey D, Cordova B, Hillman M, Kutny R, Reich N, et al. A 15-kDa interferon-induced protein is derived by COOH-terminal processing of a 17-kDa precursor. J Biol Chem 1988;263:4520–2.

Lai C, Struckhoff JJ, Schneider J, Martinez-Sobrido L, Wolff T, Garcia-Sastre A, et al. Mice lacking the ISG15 E1 enzyme UbE1L demonstrate increased susceptibility to both mouse-adapted and non-mouse-adapted influenza B virus infection. J Virol 2009;83(2):1147–51.

Lenschow DJ, Lai C, Frias-Staheli N, Giannakopoulos NV, Lutz A, Wolff T, et al. IFN stimulated gene 15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. Proc Natl Acad Sci USA 2007;104:1371–6.

Malakhov MP, Kim KI, Malakhova OA, Jacobs BS, Borden EC, Zhang DE. High-throughput immunoblotting. Ubiquitiin-like protein ISG15 modifies key regulators of signal transduction. J Biol Chem 2003;278:16608–13.

Malakhova OA, Kim KI, Luo JK, Zou W, Kumar KG, Fuchs SY, et al. UBP43 is a novel regulator of interferon signaling independent of its ISG15 isopeptidase activity. EMBO J 2006;25(11):2358–67.

Okumura F, Zou W, Zhang DE. ISG15 modification of the eIF4E cognate 4EHP enhances cap structure–binding activity of 4EHP. Genes Dev 2007;21:255–60.

Osiak A, Utermohlen O, Niendorf S, Horak I, Knobeloch KP. ISG15, an interferon-stimulated ubiquitin-like protein, is not essential for STAT1 signaling and

responses against vesicular stomatitis and lymphocytic choriomeningitis virus. Mol Cell Biol 2005;25(15):6338–45.
Randall G, Chen L, Panis M, Fischer AK, Lindenbach BD, Sun J, et al. Silencing of USP18 potentiates the antiviral activity of interferon against hepatitis C virus infection. Gastroenterology 2006;131(5):1584–91.
Ritchie KJ, Hahn CS, Kim KI, Yan M, Rosario D, Li L, et al. Role of ISG15 protease UBP43 (USP18) in innate immunity to viral infection. Nat Med 2004;10:1374–8.
Ritchie KJ, Malakhov MP, Hetherington CJ, Zhou L, Little MT, Malakhova OA, et al. Dysregulation of protein modification by ISG15 results in brain cell injury. Genes Dev 2002;16(17):2207–12.
Schwer H, Liu LQ, Zhou L, Little MT, Pan Z, Hetherington CJ, et al. Cloning and characterization of a novel human ubiquitin-specific protease, a homologue of murine UBP43 (Usp18). Genomics 2000;65:44–52.
Takeuchi T, Iwahara S, Saeki Y, Sasajima H, Yokosawa H. Link between the ubiquitin conjugation system and the ISG15 conjugation system: ISG15 conjugation to the UbcH6 ubiquitin E2 enzyme. J Biochem 2005;138:711–9.
Takeuchi T, Kobayashi T, Tamura S, Yokosawa H. Negative regulation of protein. phosphatase 2Cbeta by ISG15 conjugation. FEBS Lett 2006;580:4521–6.
VanDemark AP, Hofmann RM, Tsui C, Pickart CM, Wolberger C. Molecular insights into polyubiquitin chain assembly: crystal structure of the Mms2/Ubc13 heterodimer. Cell 2001;105:711–20.
Wong JJ, Pung YF, Sze NS, Chin KC. HERC5 is an IFN-induced HECT-type E3 protein ligase that mediates type I IFN-induced ISGylation of protein targets. Proc Natl Acad Sci USA 2006;103:10735–40.
Yoshida H, Kitamura K, Tanaka K, Omura S, Miyazaki T, Hachiya T, et al. Accelerated degradation of PML-retinoic acid receptor alpha (PML-RARA) oncoprotein by all-trans-retinoic acid in acute promyelocytic leukemia: possible role of the proteasome pathway. Cancer Res 1996;56:2945–8.
Zhao C, Beaudenon SL, Kelley ML, Waddell MB, Yuan W, Schulman BA, et al. The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for ISG15, an IFN-alpha/beta-induced ubiquitin-like protein. Proc Natl Acad Sci USA 2004;101:7578–82.
Zhao C, Denison C, Huibregtse JM, Gygi S, Krug RM. Human ISG15 conjugation targets both IFN-induced and constitutively expressed proteins functioning in diverse cellular pathways. Proc Natl Acad Sci USA 2005;102:10200–5.
Zou W, Zhang DE. The interferon-inducible ubiquitin–protein isopeptide ligase (E3) EFP also functions as an ISG15 E3 ligase. J Biol Chem 2006;281:3989–94.