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Review
Transcriptional Regulation of Antiviral Interferon-Stimulated Genes

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Interferon-stimulated genes (ISGs) are a group of gene products that coordinately combat pathogen invasions, in particular viral infections. Transcription of ISGs occurs rapidly upon pathogen invasion, and this is classically provoked via activation of the Janus kinase/signal transducer and activator of transcription (JAK–STAT) pathway, mainly by interferons (IFNs). However, a plethora of recent studies have reported a variety of non-canonical mechanisms regulating ISG transcription. These new studies are extremely important for understanding the quantitative and temporal differences in ISG transcription under specific circumstances. Because these canonical and non-canonical regulatory mechanisms are essential for defining the nature of host defense and associated detrimental proinflammatory effects, we comprehensively review the state of this rapidly evolving field and the clinical implications of recently acquired knowledge in this respect.

Host Antiviral Defense
IFN-mediated innate immune response forms a forward line of cell-autonomous defense against pathogens. Virus invasion (e.g., the presence of single-stranded RNA in endosomes or cytosolic double-stranded RNA) triggers the host cells to recognize the infection through pattern recognition receptors (PRRs), that in turn mediate the production of IFNs [1]. The thus-released IFN molecules bind to cell-surface receptors and initiate signal transduction prominently involving the Janus kinase/signal transducer and activator of transcription (JAK–STAT) pathway. This activates the transcription of hundreds of so-called ISGs that are the effectors of cell-autonomous antiviral defense. Representative and well-studied ISG members with specific or broad antiviral activities include RIG-I, MDA5, MX2, IRF1, IRF3, IRF7, IRF9, IFITM3, ISG15, and OASL [2]. ISGs act at different stages of the viral life cycle, from entry, replication, assembly to release. This leads to a remarkable antiviral state that provides adequate cellular immunity against positive-, negative-, and double-stranded RNA viruses, DNA viruses, and even intracellular bacteria and parasites.

Although the JAK–STAT pathway plays key roles in regulating ISG transcription, a far more complex cell signaling network with both canonical and non-canonical mechanisms is involved [3]. The signaling strength, kinetics, and specificity of regulatory pathways on ISG transcription are modulated at various levels by distinct mechanisms operating in conjunction. Understanding the different mechanisms of ISG transcription and how their modes of action relate to clinically used antiviral medications will provide new insights into virus–host interactions and novel avenues for antiviral drug development. Therefore, we aim to comprehensively review the classical and non-classical mechanisms regulating ISG transcription with emphasis on their clinical implications.

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Classical Mechanisms of Regulating ISG Transcription: The IFN–JAK–STAT Pathway

Upon IFN binding to its cognate cell-surface receptors, a signal is transmitted through the membrane into the cell via the JAK–STAT pathway, leading to rapid transcriptional activation of ISGs [4]. Decades of dedicated efforts have elucidated this classical regulatory network, as outlined here (Figure 1).

IFNs and Their Receptor-Dependent Regulation

Genes encoding IFNs and their receptors have been duplicated extensively throughout vertebrate evolution, indicating substantial evolutionary pressure on this system in combating pathogens [5]. Until now, >20 distinct IFN genes/proteins have been identified. Based on the type of receptor through which they signal, the multitude of different IFNs in mammalian

![Diagram of IFN signaling pathways](image)

Figure 1. The Classical Interferon (IFN) Signaling Pathways in Regulating IFN-Stimulated Gene (ISG) Transcription. The three different classes of IFNs signal through their corresponding receptor complexes, leading to phosphorylation of preassociated Janus kinases. For type I and III IFNs, the phosphorylated Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) in turn phosphorylate the receptors at specific intracellular tyrosine residues. This leads to the recruitment and phosphorylation of signal transducers and activators of transcription 1 and 2 (STAT1 and STAT2) at specific tyrosine residues. STAT1 and 2 then recruit IRF9 to form the IFN-stimulated gene factor 3 (ISGF3). For type II IFNs, the phosphorylated JAK1 and JAK2 tyrosine kinases phosphorylate the receptors on tyrosines, leading to homodimerization of STAT1. Both ISGF3 and STAT1 homodimers translocate to the nucleus for further phosphorylation at specific serine residues of STAT1, thereby achieving full activation. Consequently, ISGs are transcriptionally activated by binding of ISGF3 and STAT1 homodimers to IFN-stimulated response elements (ISREs) and γ-activated sequence (GAS) promoter elements, respectively. Conversely, specific phosphatases in the nucleus dephosphorylate STAT1 and STAT2 to avoid excessive and detrimental responses.
genome are classified into three major types: I, II, and III. In humans, type I IFNs include IFN-α (which can be further subdivided into 13 different subtypes), IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-τ, and IFN-ω1–3. All type I IFNs bind to a common cell-surface receptor, the type I IFN heterodimeric receptor complexes comprising two subunits: IFN-α receptor 1 (IFNAR1) and IFN-α receptor 2 (IFNAR2). Unlike type I IFNs, there is only one type II IFN, IFN-γ. It has no marked structural homology with type I IFNs. IFN-γ binds to a different cell-surface receptor composed of two subunits: IFNFR1 and IFNFR2. The type III IFN family comprises four members: IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B), and IFN-λ4 (frameshift variant of IL-28B). They signal through the IFN-λ receptor (IFNLR) which is composed of two subunits: IFNLR1 (IL28Rx) and IL10Rβ.

Type II IFN signaling leads to STAT1 phosphorylation, followed by homodimerization, nuclear translocation, and DNA binding at γ-activated sequence (GAS) elements located within promoter regions of IFN-γ-induced genes. While both type I and III IFN signaling activate a similar intracellular JAK–STAT pathway to generate the transcription complex, ISGF3, that transcribes ISGs, they utilize distinct receptor complexes for signaling [6]. However, IFNAR is ubiquitously expressed in all nucleated cells, whereas IFNLAR1 is only expressed on specific tissues/cells of epithelial origin [7], suggesting a selectivity of type III IFNs compared with type I IFNs.

For optimal activation, signaling through the IFN receptor complex depends on tyrosine phosphorylation, serine phosphorylation, and acetylation of IFN receptors (Table 1) [8–10]. Nevertheless, negative regulation is also essential for balancing its beneficial antiviral versus detrimental proinflammatory effects. Primarily, this is achieved by (i) phosphorylation-induced IFN receptor ubiquitination and degradation [11]; (ii) blocking the interaction between IFNAR and downstream signaling elements, such as the function of USP18, ISG15, and SOCS1 [12–16]; (iii) receptor-mediated ligand internalization/degradation [17]; and (iv) modulating cell-surface IFN receptor levels [18,19].

**JAK Kinase-Dependent Regulation**

The JAKs comprise four members, three of which (JAK1, JAK2, and TYK2) function in IFN signaling and are ubiquitously expressed [20]. They are preassociated with the corresponding IFN receptor. Upon IFN binding to the receptor, they become activated through close-proximity trans-phosphorylation (JAK1, Tyr1022,1023; JAK2, Tyr1007,1008; and TYK2, Tyr1054,1055). Subsequently, activated JAKs phosphorylate the cytoplasmic regions of the receptor, generating docking sites for SH2 domain-containing proteins, in particular STAT1 and STAT2 [21]. Activation of JAK enzymatic activity also triggers negative feedback on antiviral immunity. Phosphatases, including T cell protein tyrosine phosphatase (TCPTP), and protein tyrosine phosphatases (PTP) 1B and CD45, are the most important negative regulators [22–25]. The SOCS-1 protein also negatively regulates this process through phosphorylation-mediated proteasomal degradation of JAK [26]. The key function of JAKs in cell signaling has made them ideal targets for controlling a range of autoimmune diseases. Several JAK inhibitors have been approved by the FDA or are in clinical trials for the treatment of rheumatoid arthritis, psoriasis, inflammatory bowel disease, and ankylosing spondylitis [27].

**STAT-Dependent Regulation**

There are seven STAT members in mammals, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. STAT1 and STAT2 are the most important STATs with respect to IFN signaling [2]. In response to IFNs, STAT1 is phosphorylated on Tyr701, Ser705, and Ser727. These sites are all positively related to signaling transduction [28,29]. STAT2 acquires transcriptional activation upon tyrosine phosphorylation (Tyr690). Conversely, serine phosphorylation (Ser285) of STAT2 negatively regulates the IFN response [21,30]. Although JAKs play key
Table 1. Classical Polypeptide Modifications in the IFN–JAK–STAT Pathway.

| Modification site | Modification typea | Signal transduction | Refs |
|-------------------|-------------------|---------------------|------|
| IFNAR1            | Tyr466            | Phosphorylation     | Activation [125] |
| IFNAR1            | Tyr512 and Tyr337 | Phosphorylation     | Activation [126] |
| IFNAR1            | Ser535, Ser539    | Phosphorylation     | Inactivation [11] |
| IFNAR1            | Lys507, Lys526,   | Ubiquitination      | Inactivation [11] |
| IFNAR2            | Ser264, Ser264    | Phosphorylation     | Activation [9] |
| IFNAR2            | Lys399            | Acetylation         | Activation [9] |
| IFNGR1            | Pro287            | ND                   | Activation [127] |
| IFNGR1            | Tyr440            | Phosphorylation     | Activation [128] |
| IFNGR1            | 270L271           | ND                   | Inactivation [17] |
| IFNGR1            | Tyr441            | Phosphorylation     | Inactivation [16,128] |
| IFNGR2            | 263PPSIP267 and   | ND                   | Activation [10] |
|                   | 270IEEYL274       |                     |      |
| JAK1              | Tyr1022,1023      | Phosphorylation     | Activation [21] |
| JAK2              | Tyr1007,1008      | Phosphorylation     | Activation [21] |
| TYK2              | Tyr1054,1055      | Phosphorylation     | Activation [21] |
| STAT1             | Tyr701            | Phosphorylation     | Activation [129] |
| STAT1             | Ser727            | Phosphorylation     | Activation [129] |
| STAT1             | Ser708            | Phosphorylation     | Activation [28] |
| STAT1             | Lys703            | SUMO-1 binding      | Inactivation [47] |
| STAT2             | Tyr690            | Phosphorylation     | Activation [30] |
| STAT2             | Ser287            | Phosphorylation     | Inactivation [30] |

*aND, not determined.

role in STAT1 phosphorylation and activation, other cellular factors are also required. Tyrosine kinase non-receptor 1 (TNK1) and retinoic acid-inducible gene I (RIG-I) potentiate dual phosphorylation of STAT1 at Tyr701 and Ser727 [31–33], whereas nuclear cyclin-dependent kinase 8 (CDK8) phosphorylates Ser727 of STAT1 [34,35]. Protein kinase C family members, PKC-δ or PKC-ε, mediate phosphorylation of STAT1 on Ser727 (no effect on STAT1 tyrosine phosphorylation) via the upstream phosphatidylinositol 3-kinase (PI3K)–Akt pathway [36–39]. Interestingly, stress signals can also induce phosphorylation of STAT1 (Ser727) via the p38–MAPK pathway [40]. Because p38–MAP kinase inhibitors are well-tolerated and safe for humans, it is thus tempting to speculate that such inhibitors might be used to mitigate proinflammatory effects following IFN-γ therapy [41].

Evidently, phosphatase-dependent STAT1 dephosphorylation constitutes an important negative-regulatory event that is central in titrating the IFN response. The functional phosphatases include SHP-2 [42,43], the nuclear isoform of TCPTP, TC45 [44], and SHPPT1 [45]. Phosphatase dysregulation has been reported in cancers and autoimmune disorders, thus representing potential therapeutic targets [46]. A small ubiquitin-related modifier 1 (SUMO-1) was also reported to conjugate at Lys703 of STAT1 to inhibit signal transduction [47]. Thus, a plethora of molecular mechanisms can balance the IFN response through acting on STAT1.

IRF9 is a major DNA binding component of the ISGF3 complex. IRF9 alone binds to DNA and recognizes the specific promoter elements denoted as IFN-stimulated response elements (ISRE), but has no transcriptional activity. Upon DNA binding, IRF9 provides specific protein–DNA interaction sites for STAT1 and STAT2. Activated STAT1 and STAT2 bind to the ISRE region together with IRF9 to exert strong pro-transcriptional activity [48]. Theoretically, IRF9 (as
part of the ISGF3 complex) is only involved in regulating ISG transcription downstream of type I and III IFN signaling. However, IFN-γ-induced ISG activation and the antiviral state were severely impaired in the absence of IRF9, indicating that IRF9 may also be involved in type II IFN signaling [49,50]. More interestingly, IFN-γ pretreatment induces high levels of IRF9, which serves as an important subunit of the latent precursor to ISGF3. In this way, IFN-α and IFN-γ synergize to induce the formation of ISGF3 complex, leading to much stronger ISG transcription [51].

**Regulation of ISGs at the Transcriptional Level**

In the case of type I and III IFNs, ISGF3 is the predominant transcriptional factor binding to ISREs within the promoter region of ISGs; whereas for type II IFN homodimers or heterodimers of STATs are the determinant of binding to GAS elements. However, this is a simplified model and other regulatory elements are also involved (Figure 2).

**Chromatin Modulators**

Histone octamers bind to DNA and organize chromatin into higher-order nucleosomes, prohibiting transcription factor binding and gene expression [52]. As a consequence, the induction of ISGs by IFNs requires chromatin remodeling. The condensed chromatin needs to be transformed into a more relaxed structure. In humans, the nucleosome remodeling complexes BAF and PBAF prime ISG promoters by utilizing ATP-derived energy to maintain chromatin in a constitutively open conformation, allowing fast and potent induction of ISGs after IFN exposure [53–56]. Histone acetylation and deacetylation are also essential in chromatin modulation. These reactions are typically catalyzed by enzymes with histone acetyltransferase (HAT) or histone deacetylase (HDAC) activity. HAT activity transforms chromatin into a more relaxed structure, while HDAC activity organizes chromatin into higher-order nucleosomes. Therefore, the HAT family members, including p300/CBP and GCN5, are essential for

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**Figure 2. Transcriptional Regulation of IFN-Stimulated Genes (ISGs) Involves Chromatin Remodeling and Various Coactivators and Corepressors.** Upon IFN stimulation, IFN-stimulated gene factor 3 (ISGF3) or STAT1 homodimers bind to ISG promoter regions, recruiting various chromatin remodeling factors and transcriptional coactivators. These factors include the nucleosome remodeling complexes BAF and PBAF, p300/CBP and GCN5 histone acetyltransferase (HAT), histone deacetylase (HDAC), minichromosome maintenance 3 and 5 (MCM3 and MCM5), N-Myc interactor (NMI), and DRIP150 (a subunit of the multimeric mediator coactivator complex). Consequently, the condensed chromatin transforms into a more relaxed structure to facilitate the transcription of ISGs. Conversely, corepressor factors can inhibit ISG transcription either via the facilitation of a closed chromatin configuration or by interfering with the recruitment of STAT1 or ISGF3 to ISG promoters. Abbreviation: Pol II, RNA polymerase II.

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transcriptional activation of ISGs [57,58]. HATs are positive regulators of transcription in general. However, HDAC activity is also essential for transcriptional induction of ISGs [59–64]. HDAC activity has been reported to be required for recruiting RNA polymerase II to the promoters of ISGs [65], although how HDACs regulate the transcriptional activation of ISG remains unclear. In addition, the FOXO3 and PI3K/AKT pathways coordinately modulate chromatin structure. FOXO3, together with nuclear corepressor 2 (NCOR2) and HDAC3, forms a ternary complex to facilitate a closed chromatin structure, thereby limiting ISG transcription under basal conditions. However, type I IFN can activate the PI3 K/AKT pathway, which in turn leads to FOXO3 degradation and ISG transcription [66].

Coactivators and Corepressors

Particular coactivators or corepressors mediate the transcription of ISGs via the interaction with ISGF3 or STAT1 homodimers. The coactivators, such as MCM5 (minichromosome maintenance) and MCM3 protein complex [67,68], N-Myc interactor (NMI) [69], and Drp1P150 [70], facilitate the transcriptional activation of ISGs. Conversely, corepressors, such as TAF-1 [71] and the protein inhibitor of activated STAT proteins (PIAS1 and PIASy [72,73]), suppress the formation of transcription complexes on ISG promoters to limit transcription. Recently, four previously unrecognized regulatory factors (ETV6, ATF3, LYN, and TBK1) of ISG transcription have been identified [74]. These efforts have led to a more comprehensive understanding of ISG transcription.

Non-canonical Regulation of ISG Transcription

All three types of IFNs signal through the JAK–STAT pathway to elicit antiviral activity. Nevertheless, type II IFN is thought to do so only through STAT1 homodimers, whereas type I and III IFNs activate both STAT1 and STAT2 to form ISGF3 together with IRF9. However, accumulating evidence highlights a far more complex process of activation and function beyond this classical theory. The heterogeneity of the regulatory mechanisms of ISG transcription has been recently highlighted. A substantial fraction of these cascades have little or no link to STAT1/2 and ISGF3, paralleling the existence of non-canonical mechanisms outside the JAK–STAT axis [74]. We review here both JAK–STAT axis-dependent and -independent non-canonical mechanisms of ISG transcription (Figure 3).

Non-canonical ISGF3 Complex

To date, three different forms of non-canonical ISGF3 complexes have been identified, including ISGF3III, the STAT2–IRF9 complex, and unphosphorylated ISGF3 (U-ISGF3). IFN-γ treatment has been reported to lead to the formation of a new ISGF3 complex (ISGF3III) containing phosphorylated STAT1, unphosphorylated STAT2, and IRF9 [75]. In the absence of STAT1, STAT2 was found to interact with IRF9 to form an ISGF3-like complex to mediate specific ISG transcription [76]. Finally, following continuous exposure to low levels of exogenous IFNs, U-ISGF3 formed by IFN-induced IRF9 and unphosphorylated STAT1 and STAT2 can lead to increased expression of a subset of ISGs [77,78].

STAT5–CrkL Complex

Apart from STAT1 and STAT2, STAT5 is also involved in type I IFN-induced ISG transcription. STAT5 interacts constitutively with IFN receptor-associated TYK-2. Upon type I IFN stimulation, STAT5 is phosphorylated on both tyrosine and serine sites, thus acting as a docking site for the SH2 domain of CrkL. CrkL and STAT5 then form a complex that translocates to the nucleus and binds to GAS elements to activate type I IFN-dependent gene transcription [3,79].

IRFs

IRF1 has been shown to function as a transcription factor. The DNA sequences (IRF-E site) recognized by IRF1 overlap with the ISRE, and in this way IRF1 induces a subset of ISGs. IRF1
can also enhance the levels of both total and phosphorylated STAT1 to amplify ISG transcription via the JAK–STAT pathway [80]. Conversely, IRF2 binds to the same IRF-E site to repress IRF1-induced transcription [81,82]. Upon virus infection, IRF3 is activated and cooperates with NF-κB and ATF-2/c-Jun to form a transcriptionally active enhanceosome complex on the IFN-β promoter. Newly synthesized IFN binds to cognate receptors to activate ISG transcription via the JAK–STAT pathway. Importantly, IRF3 has also been reported to directly induce a subset of ISGs in an IFN-independent manner through ISREs in their promoters [83,84].

Crossregulation between TNF and IFN Signaling
It is well documented that, when combined with TNF-α, type I or II IFN operates cooperatively to induce antiviral ISG expression, and TNF-α and IFN exert synergistic antiviral effects [85–88]. TNF-α has been reported to inhibit hepatitis C virus (HCV) infection-caused degradation of IFNAR2, thus maintaining IFN signaling and ISG expression [88]. TNF-α alone can already moderately induce the transcription of a subset of ISGs [85,86]. This is mainly through the NF-κB protein complex, a key downstream element of TNF-α signaling. This may explain the documented antiviral activity of TNF-α on different viruses [87,89–91].

Figure 3. Non-canonical Mechanisms Regulating ISG Transcription. Non-canonical mechanisms both within and outside the IFN–JAK–STAT axis are summarized. Together with canonical mechanisms, they coordinately regulate ISG transcription, thus defining cellular defense status against pathogen invasion.
Rac1/p38 Pathway
Rac1/p38 MAP kinase signaling regulates IFN induced ISG transcription. Type I IFN treatment results in activation of Rac1 and its downstream effectors including MAP kinase kinase 3 (MKK3), MAP kinase kinase 6 (MKK6) [92,93], and cytosolic phospholipase A2 [94,95]. In turn, these events provoke phosphorylation and activation of the p38 MAP kinase, an important mediator of the inflammatory response [96]. p38 MAP kinase activation leads to downstream MapKapK-2 and MapKapK-3 activation, contributing to type I IFN-dependent transcriptional regulation of ISGs. However, Rac1/p38 MAP kinase signaling is not required for IFN-dependent phosphorylation of STAT1 at both sites (Ser727 and Tyr701) and has no impact on the formation of the ISGF3 complex [97,98]. Histone phosphorylation and chromatin remodeling are possible mechanisms employed by this cascade [97]. Many immune-relevant gene products are subject to post-transcriptional regulation by this signaling [99], but ISGs have not been investigated in this respect.

IFN-γ-Activated Response Element (GATE)
In response to IFN-γ, two factors bind to a unique IFN-γ-activated response element known as GATE – these are the CCAAT/enhancer binding protein C/EBP-β and the GATE binding factor GBF-1. MEK1, ERK1, and ERK2 are the upstream kinases necessary to activate C/EBP-β in response to IFN-γ [100]. This novel IFN-γ-activated pathway promotes ISG expression in a STAT1- but not JAK1-dependent manner.

Nucleotide Synthesis Inhibitor
Purine and pyrimidine nucleotides are the major cellular energy carriers and are subunits of nucleic acids. Nucleotides can be synthesized de novo through a series of enzymatic reactions or are recycled through salvage pathways. Interestingly, purine and pyrimidine synthesis inhibitors (such as ribavirin, mycophenolic acid, and brequinar) can efficiently induce ISG expression and exert strong and broad antiviral responses [101–103]. However, this process is independent of the classical JAK–STAT cascade, suggesting a non-canonical mechanism that is independent of IFNs [104]. Ribavirin, an inhibitor of the IMPDH enzyme, was shown to reset a subset of ISG promoters to a ‘ready to be activated’ state, thus potentiating ISG activation [105]. However, crosstalk between nucleotide synthesis and the innate immune response remains to be further elucidated.

Retinoic Acid (RA)
RA is a metabolite of vitamin A that mediates the functions of vitamin A in growth and development. RA activates transcriptional via heterodimers of retinoic acid receptors (RAR) and retinoid X receptors (RXR), and these bind to regions in promoters known as retinoic acid response elements (RAREs). Numerous studies have reported antiviral activities of RA against a variety of pathogens [106,107]. Interestingly, intracellular RA increases ISG expression at basal levels and augments ISG induction in response to IFNs [108]. This is consistent with the clinical observation that RA enhances the response to IFN-based antiviral therapy [107,109]. Strikingly, a bioinformatics study showed that most ISG regulatory regions contain RARE sequences [108]. This indicates that RA can induce transcriptional activation of these ISGs containing RAREs, facilitating the binding of additional transcription factors to the promoters of these ISGs. Consequently, RA initiates and works synergistically with IFNs to induce ISG expression.

IFNs and ISGs: Clinical Implications and Future Perspective
IFNs have been used in various clinical settings to counteract pathogen-related diseases. Because of its robust and broad antiviral activity, IFN-α has represented the standard treatment for chronic hepatitis B virus (HBV) or HCV infections for decades. Its application also extends to other virus infections as an off-label treatment, for example hepatitis E virus [110] and severe acute respiratory syndrome [111]. IFN-λ has been shown to play a crucial role in cancer,
autoimmune disease, and viral infections [112]. The antitumor and anti-infection activities of IFN-γ have been comprehensively evaluated and used in a variety of clinical indications. It has been approved by FDA to treat chronic granulomatous disease and osteopetrosis, and is experimentally used for the treatment of idiopathic pulmonary fibrosis and Friedrich’s ataxia [113]. However, IFN-γ has not been successful in treating viral infections [114,115]. IFN-λ has shown specific antiviral activity in both chronic HBV and HCV patients; although its efficacy was not superior compared to IFN-α therapy, IFN-λ had more limited side effects [116,117]. This is because IFNAR1 has a more restricted tissue-specific pattern of expression. IFN-λ has also been shown to determine the intestinal epithelial antiviral host defense against rotavirus infection. It acts synergistically with IL-22 for the induction of ISGs, and eventually controls rotavirus infection in animal models [118,119]. Thus, IFN-λ might be an attractive option for the treatment of many viral infections. Although the clinical application of IFNs, in particular for HCV, will be limited because of the recent launch of direct-acting antiviral agents, it may extend to other devastating viral diseases such as Ebola, Zika, or dengue virus infections.

Mechanistically, for all three types of IFNs, ISGs are the ultimate antiviral effectors. Recent studies on the function of individual ISGs indicate that different viruses are targeted by unique sets of ISGs. Some ISGs possess broad antiviral properties whereas others have specific antiviral effects [120]. Thus, characterization of individual ISGs with respect to their antiviral spectrum or specificity provides new avenues for improving current antiviral therapies. Interestingly, several ISGs have been reported to paradoxically enhance the replication of certain viruses, illustrating the complexity of the network of mutual interaction between ISGs and viruses [120]. In preclinical or clinical studies, the expression patterns of some specific ISGs have been identified as biomarkers to predict treatment responses, disease progression, or outcomes in both infectious (e.g., HCV and HIV infections) [121–123] and non-infectious human diseases (e.g., Aicardi-Goutières syndrome and systemic lupus erythematosus) [124,125]. Some ISGs (e.g., TLR3, TLR7, RIG-I, and MDA5) belong to the class of PRRs. Given their key roles in innate immune responses, there is growing interest in targeting PRRs for the prevention and treatment of cancer, autoimmune diseases, and infections. Specific activators are now undergoing preclinical and clinical evaluation for safety and efficacy [126]. With regards to the adverse effects of IFNs in the clinic, ISG-based antiviral strategies could be the next promising frontier in drug discovery.

Concluding Remarks

Decades of research have shaped a picture of the complex networks regulating ISG transcription. These include both canonical and non-canonical mechanisms within and outside the IFN–JAK–STAT axis, coordinately defining the cellular defense status against pathogen invasion. We expect that the spectrum of new elements involved in both canonical and non-canonical regulation of ISG transcription will continue to grow, and their mechanism-of-actions will be further clarified (see Outstanding Questions). Because of their importance in clinical implication, this knowledge is highly relevant in guiding the development of new therapies that promote the eradication of severe pathogen infections while avoiding autoimmune diseases and toxic effects to the host.

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