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A dive into the complexity of type I interferon antiviral functions

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COMMENTARY ON:

A diverse range of gene products are effectors of the type I interferon antiviral response. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM. Nature. 2011 Apr 28;472(7344):481–485. Copyright (2011). Abstract reprinted by permission from Macmillan Publishers Ltd.

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Abstract: *The type I interferon response protects cells against invading viral pathogens. The cellular factors that mediate this defense are the products of interferon-stimulated genes (ISGs). Although hundreds of ISGs have been identified since their discovery more than 25 years ago, only a few have been characterized with respect to antiviral activity. For most ISG products, little is known about their antiviral potential, their target specificity, and their mechanisms of action. Using an overexpression screening approach, here we show that different viruses are targeted by unique sets of ISGs. We find that each viral species is susceptible to multiple antiviral genes, which together encompass a range of inhibitory activities. To conduct the screen, more than 380 human ISGs were tested for their ability to inhibit the replication of several important human and animal viruses, including hepatitis C virus, yellow fever virus, West Nile virus, chikungunya virus, Venezuelan equine encephalitis virus, and human immunodeficiency virus type-1. Broadly acting effectors included IRF1, C6orf150 (also known as MB21D1), HPSE, RIG-I (also known as DDX58), MDA5 (also known as IFIH1), and IFITM3, whereas more targeted antiviral specificity was observed with DDX60, IFI44L, IFI6, IFITM2, MAP3K14, MOV10, NAMPT (also known as PBEF1), OASL, RTP4, TREX1, and UNC84B (also known as SUN2). Combined expression of pairs of ISGs showed additive antiviral effects similar to those of moderate type I interferon doses. Mechanistic studies uncovered a common theme of translational inhibition for numerous effectors. Several ISGs, including ADAR, FAM46C, LY6E, and MCOLN2, enhanced the replication of certain viruses, highlighting another layer of complexity in the highly pleiotropic type I interferon system.*

Introduction

Type I interferons are a family of major innate immune cytokines produced by host cells in response to viral infection [1]. Since their discovery 50 years ago, fundamental and biomedical research has greatly improved our understanding of their molecular mechanisms of action, and led to the development of the first "cytokine-based" therapy in the 70s, now licensed worldwide for viral disease, malignant and even immune disorders [1,2].

Interferon remains the therapeutic backbone of chronic hepatitis C. The standard of care, in HCV genotype 1 infected patients, is the addition of direct-acting antivirals (DAAs) with a protease inhibitor (telaprevir or boceprevir) to pegylated interferon plus ribavirin [3].

The type I interferon family is composed of 5 members in humans: the well described IFNα and IFNβ, along with IFNκ, IFNε, IFNω that are less characterized, and more tissue targeted [4,5].

There are 13 IFNα and one IFNβ isoforms, all acting through a unique ubiquitous heterodimeric receptor IFNAR1/IFNAR2. Downstream signaling pathways have been extensively described: phosphorylation of tyrosine kinases JAK1 and TYK2 results in the recruitment of STAT1 and STAT2 which migrate into the nucleus and associate with IFN regulatory factor 9 (IRF9) to form the IFN-stimulated gene factor 3 (ISGF3). This complex then activates the transcription of all the IFN Stimulated Genes (ISGs), which mediate diverse cellular effects in the infected cell. The study of highly induced ISGs (MX1, OAS, dsRNA-activated protein kinase PKR) led to fundamental discoveries concerning the translational control and regulation of RNA stability [6].

Unresolved questions

The function of many ISGs, however, remains unknown, limiting our ability to manipulate IFN in a rational manner and predict its therapeutic and side effects. In particular, it is not known

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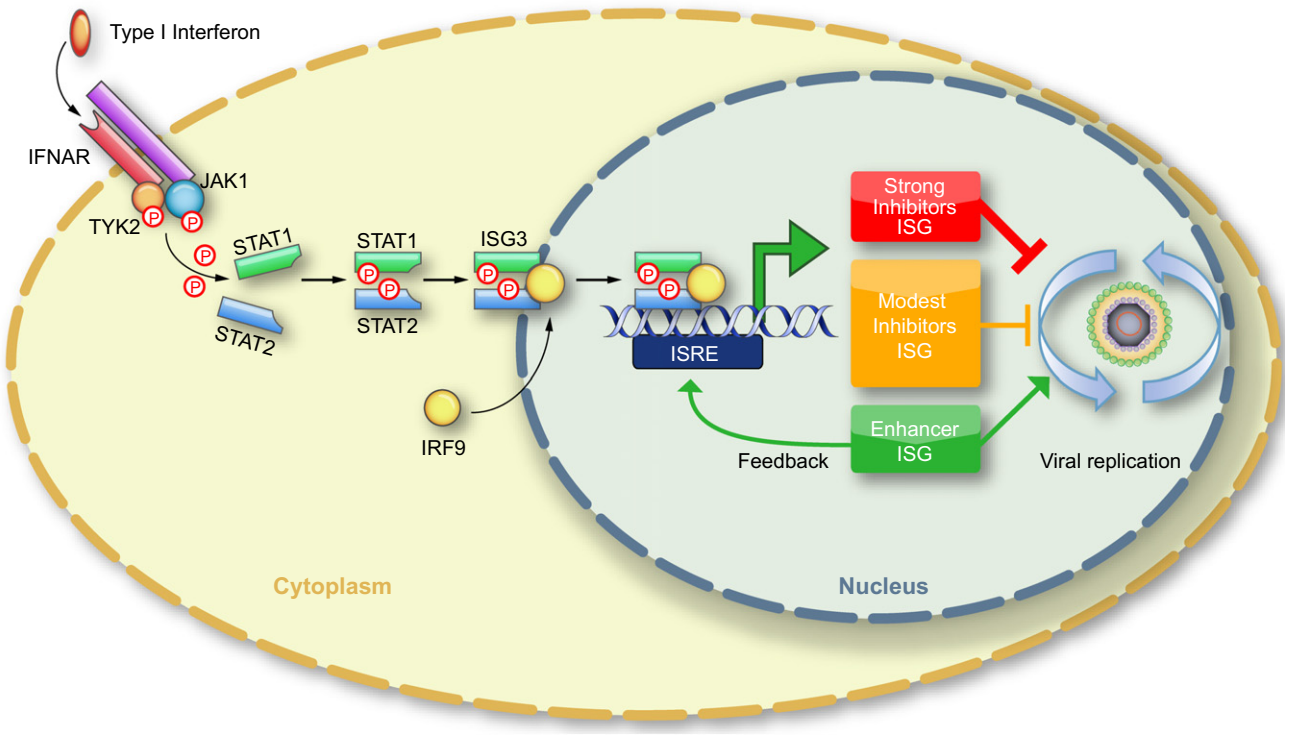

Fig. 1. New view of ISG's function in viral replication. Interferon stimulated genes (ISG) can be divided in 3 groups: strong inhibitors, modest inhibitors or enhancers. ISGs use multiple strategies to inhibit viral replication: either by targeting specific phase of viral replication (e.g. primary translational inhibition) or/and by potentiating IFN response by a positive feedback loop. IFNAR, Interferon receptor; ISRE, Interferon response stimulating elements, IRF9, Interferon response factor 9.

whether all ISGs share the same antiviral potential and/or mechanism of action.

In the issue of April 2011 of *Nature*, Schoggins and colleagues succeeded in answering these questions [7]. They proposed a new model to analyze the antiviral function of ISG in a systematic and large-scale manner. They developed a cell-based assay using a lentiviral vector co-expressing an ISG and a red fluorescent protein, TagRFP, in order to overexpress the ISG in different cell types. They subsequently challenged these cells with different green fluorescent proteins (GFP)-expressing viruses (including HCV) to assess the inhibitory capacity of all the ISG on viral replication by flow cytometry.

Interestingly, they identified 3 main categories of ISGs for each virus: a small group with strong inhibitory effect that probably has a feedback into the IFN-mediated signaling pathway; a major group with moderate inhibitory functions, and a small group that surprisingly enhances viral replication. Moreover, the use of combinations of two inhibitory ISGs increased the inhibition to 90% for HCV, HIV, and yellow fever virus replication.

Nucleic acid binding, hydrolase, and helicase activities were the main molecular functions of the ISG. The authors then investigated the potential mechanism of action of selected inhibitory ISGs. Translational inhibition appears to be a common mechanism of ISG-mediated antiviral effect which correlates with percent of inhibition. In the case of HCV, IRF1, IRF2, IRF7 MDA5, RIG-I, MAP3K14, and OASL were the most efficient ISG and inhibited primary translation by 25–70% after 4 h of infection. None of them was able to significantly impair viral entry into the cell.

These results support the concept that the downstream effectors of Type I interferon exploit multiple strategies to block viral replication at an early stage, in an additive manner. Some of the ISGs, however, have the paradoxical effect of enhancing viral replication at least in this experimental model.

Novelty of this article

This is the first study on IFN downstream effectors to screen such large numbers of ISGs (380) in a systematic manner. Moreover, the reported findings point out new differences between ISGs in terms of viral replication and mechanism of action, which change our current view of ISG function (Fig. 1).

Some ISGs have broad effects on different viruses (IRF1, C6orf150, RIG-1, MDA5) whereas others are more target-specific (IFI44l, IFI6, OASL, IFIT3M). Even if they don't share the same mechanism of action, they can have additive effects to maximize viral inhibition. Capacity of viral inhibition varies among ISGs, and the authors showed for the first time that few of them could indeed enhance viral replication. It would be interesting now to test the ISGs on other viruses in order to have a complete view of ISGs functions.

Perspectives, unanswered questions

An important question remains whether the *in vitro* over-expression of the ISGs reflects *in vivo* expression. It is crucial to validate the targeted set of ISGs on *in vivo* or *ex vivo* samples. To date, several studies on liver gene expression in chronic hepatitis C have already identified a type I interferon signature (MX1, OAS1, IFI27, viperin) [8–11]. None of these molecules appear to have a

strong "inhibitory potential" for HCV replication according to the Shoggins study. Interestingly, in chronic hepatitis C, prior to the initiation of treatment, gene expression profiles differ between non-responders and responders. The most notable changes in gene expression are mainly observed in the IFN stimulated genes [12]. A two-gene signature (*IFI27* and *CXCL9*) was able to predict treatment response. Interestingly, the baseline liver levels of expression of IFN stimulated genes were higher in non-responders than in sustained virological responders. The failure to respond to exogenous PEG-IFN in non-responders could indicate a blunted response to IFN. This suggests that IFN stimulated genes are already maximally induced in non-responders.

Furthermore, it seems also that some ISGs can enhance HCV replication but these were not described in details. Another paradoxical finding is that HCV through NS3-4A expression may inhibit the RIG-1 and MDA pathway that was found to be the most efficient inhibitor of HCV replication [13]. Follow up studies are necessary to extend and validate the Schoggins' findings in complementary model systems, as well as on patient material.

Conclusions

In their study, Schoggins and colleagues bring new insight into the effector mechanisms of type I IFN responses. The understanding of antiviral mechanisms of IFN is crucial for the discovery of new treatment biomarkers for efficacy and toxicity. Moreover, there is a need for improvement of IFN therapy with regard to the clinical side effect and viral resistance. Focus on specific sets of ISGs could lead to the development of a more targeted therapy, by specifically inhibiting viral replication, while diminishing the side effects observed with type I IFNs. Future investigation and therapeutic clinical trials will be crucial to validate the potential of using ISGs *in vivo*.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding of conflict of interest with respect to this manuscript.

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