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IQGAP2 is a novel interferon-alpha antiviral effector gene acting nonconventionally through the NF- κ B pathway

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Abstract

Background & Aims—Type I interferons (IFN) provide the first line of defense against invading pathogens but its mechanism of action is still not well understood. Using unbiased genome-wide siRNA screens, we recently identified IQ-motif containing GTPase activating protein 2 (IQGAP2), a tumor suppressor predominantly expressed in the liver, as a novel gene putatively required for IFN antiviral response against hepatitis C virus (HCV) infection. Here we sought to characterize IQGAP2 role in IFN response.

Methods—We used transient siRNA knock-down strategy in hepatic cell lines highly permissive to JFH1 strain of HCV infection.

Results—We found that IQGAP2 acts downstream of IFN binding to its receptor, and independently of the JAK-STAT pathway, by physically interacting with RelA (also known as p65), a subunit of the NF- κ B transcription factor. Interestingly, our data reveal a mechanism distinct from the well-characterised role of NF- κ B in IFN production. Indeed, IFN alone was sufficient to stimulate NF- κ B-dependent transcription in the absence of viral infection. Finally, both IQGAP2 and RelA were required for the induction by IFN of a subset of Interferon Stimulated Genes (ISG) with known antiviral properties.

Conclusions—Our data identify a novel function for IQGAP2 in IFN antiviral response in hepatoma cells. We demonstrate the involvement of IQGAP2 in regulating ISG induction by IFN in an NF- κ B-dependent manner. The IQGAP2 pathway may provide new targets for antiviral strategies in the liver, and may have a wider therapeutic implication in other disease pathogenesis driven by NF- κ B activation.

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Disclosures

The authors disclose no conflicts.

Author Contributions

C.B. and R.T.C. designed research; C.B., S.S., and V.Y. performed research and analyzed data; E.A.K.S., S.C., and J.H. contributed to technical and material support; E.A.K.S., J.H., C.C.S., N.A., and W.L. contributed to the critical revision of the manuscript for important intellectual content; and C.B. and R.T.C. wrote the paper.

Keywords

Hepatitis C Virus (HCV); antiviral response; IQGAP protein; Interferon (IFN)

Introduction

Hepatitis C virus (HCV) is an enveloped, positive-stranded RNA virus from the genus Hepacivirus of the *Flaviviridae* family.^{1,2} HCV infection leads to chronic infection in 75% of cases. Over 170 million people are estimated to be chronically infected worldwide, making HCV the leading indication for liver transplantation in developed countries. Type I interferons (IFN) provide the host's first line of defense against invading viral pathogens and are critically important in HCV control. Although IFN has been the backbone treatment for HCV for over two decades, significant gaps remain in our understanding of the basic biology of its antiviral response against HCV.

IFN is a secreted cytokine that acts in an autocrine and paracrine manner to regulate antiviral responses, enhance innate and adaptive immune responses, and modulate normal and tumor cell survival and death.³ In HCV-infected hepatocytes, pattern recognition receptors (PRR) such as the dsRNA sensors retinoic-acid-inducible gene I (RIG-I) and Toll-like receptor 3 (TLR3) are triggered and activate a number of transcription factors, including interferon regulatory factor 3 (IRF3) and nuclear factor κ B (NF- κ B), leading to subsequent induction of IFN- α , as well as proinflammatory cytokines and chemokines.⁴ Secreted IFN- α binds to its receptor IFNAR and triggers the JAK-STAT signaling pathway, leading to the activation of transcription factor ISGF3, comprised of STAT1, STAT2 and IRF9. This transcription factor stimulates IFN-sensitive responsive element (ISRE)-dependent transcription, directing the expression of over 300 IFN-stimulated genes (ISGs). Collectively, these ISGs mediate the biological effects of IFN, inhibiting all steps of the viral cycle and protecting neighboring uninfected cells from incoming viral progeny.³

In recent years, evidence has accumulated that beyond JAK-STAT, other signaling pathways, such as MAPK and PI3K/Akt, play an essential role in the type I IFN response by modulating ISG expression at either the transcriptional or translational level.⁵ Using an unbiased genome-wide siRNA screen, we recently identified 100 novel putative genes required by IFN to induce an antiviral state protective against HCV infection.⁶ Unlike ISGs, most of these genes are not transcriptionally upregulated by IFN and thus were defined as IFN effector genes (IEGs).

Among these IEGs is IQ-motif containing GTPase activating protein (IQGAP)-2, a large cytoplasmic scaffold protein predominantly expressed in the liver.^{6,7} IQGAP2 is, which is also found in the kidney and in platelets.⁸ IQGAP2 is defined as a tumor suppressor, since its expression is decreased in human hepatocellular carcinoma (HCC),^{9,10} and IQGAP2 knock-out mice develop HCC.^{11,12} IQGAP2-null mice also have impaired uptake of long-chain fatty acids, altered metabolic networks and enhanced insulin sensitivity, indicating a role for IQGAP2 in metabolism.^{12,13} IQGAP2 has also been described to promote actin polarization and influence cell motility, cell-cell adhesion and membrane ruffling.¹⁴ Two other IQGAP family members have been found in the liver: IQGAP1 is an oncogene up-

regulated in HCC,⁷ and IQGAP3 promotes proliferation in the liver,¹⁵ and is also overexpressed in HCC.⁷ IQGAP1 is ubiquitous and the best characterized member. A hundred interacting proteins have been identified, which regulate diverse biological and pathological processes such as cell-cell adhesion, cell migration, endocytosis, cellular signaling, cytoskeleton and tumorigenesis.¹⁶ Moreover IQGAP1 has been shown to facilitate bacterial invasion,¹⁷ and infection or virulence of a number of viruses from different families, including Marburg virus,¹⁸ Ebola virus,¹⁹ Moloney murine leukemia virus,²⁰ and Classical Swine Fever Virus.²¹ IQGAP2 remains less well characterized, and therefore our aim was to perform a detailed functional analysis of IQGAP2 and its role in antiviral responses.

Materials and Methods

Cell lines, viral infection and reagents

Huh7.5.1, Huh7.5.1/pNF- κ B-GFP, Huh7/CD81^{High} and HEK-293T cells were grown in DMEM medium with 10% heat-inactivated fetal bovine serum (FBS). Subconfluent cells were inoculated with JFH1 strain of HCV. Inoculum was replaced after an adsorption period of 4 hours with fresh medium. Human IFN- α 2a, from PBL Interferon source (11101-2 and 11100-1), were used at a final concentration of 100 IU/ml in Huh7.5.1 and Huh7.5.1/pNF- κ B-GFP cells or 10 IU/ml in Huh7/CD81^{High}.

siRNA transfection

Cells were transfected with siRNAs as described in our previous study.⁶

Assessment of percentage infected cells

The percentage of HCV-infected cells was determined as described in our previous study.⁶

Assessment of ISRE promoter activity

ISRE promoter activity was measured as described in our previous study.²² ISRE induction was calculated as the ratio relative or Firefly over Renilla luciferase activities and then normalized to mock-treated Huh7.5.1 transfected with NT siRNA set as 1.

Assessment of NF- κ B promoter activity

Huh7.5.1 cells were stably transduced with a lentivirus expressing the GFP protein under the NF- κ B promoter to generate Huh7.5.1/pNF- κ B-GFP cells. GFP fluorescence was measured with a FACScan machine (Becton Dickinson) and analyzed with Cellquest software (Becton Dickinson). Images of PFA-fixed cells were acquired with the EVOS[®] FL Cell Imaging System (Invitrogen).

Statistical analysis

Data are expressed as means $\pm$ the standard error of the mean for at least three independent experiments done in duplicate. A 2-tailed Student's *t* test was used to compare experimental conditions and controls. A *P* value of < 0.05 was considered significant, indicated by a “*”.

Results

IQGAP2 mediates HCV lifecycle inhibition by IFN

We first confirmed that IQGAP2 is required for HCV inhibition by IFN in Huh7.5.1 human hepatoma cells.⁶ Transfection of Huh7.5.1 cells with siRNA against IQGAP2 led to a significant (80%) knockdown of IQGAP2 expression (Figure 1A), without a detectable effect on cell viability, as determined by cellular ATP content (Figure 1B). We then confirmed that IQGAP2 silencing rescued HCV infection from IFN-mediated suppression. Cells were transfected with siRNA against IQGAP2, or IFNAR as a positive control. An antiviral state was induced by treatment with 100 IU/ml IFN- α for 24 hours before infection with HCV at a multiplicity of infection (MOI) of 1 TCID₅₀/cell. Two days post-infection (p.i.), the percentage of HCV-infected cells was assessed by immunostaining for HCV Core protein. All IQGAP2-siRNAs significantly rescued HCV infection from IFN (Figure 1C). IQGAP2-siRNA #4 was the most potent siRNA for IQGAP2 silencing (Figure 1A) and HCV rescue from IFN (Figure 1C), and hence was selected for use in subsequent experiments. We also evaluated the effect of IQGAP2 silencing on the kinetics of HCV growth in Huh7.5.1 cells pre-treated with IFN. IQGAP2 silencing led to a significant increase in intracellular HCV RNA levels at 48 and 72 hours p.i. (Figure 1D) and extracellular viral titers at 24 and 48 hours p.i., reaching a plateau at 48 hours p.i. (Figure 1E). These results confirm IQGAP2's contribution to IFN's anti-HCV activity.

Huh7.5.1 cells are derived from Huh7 cells cured from HCV replicon by prolonged IFN treatment,²³ that results in an increased permissiveness for HCV associated with a defective interferon response.^{24,25} We verified that IQGAP2's role in IFN response is not dependent on defects specific to Huh7.5.1 cells, by confirming our results in Huh7/CD81^{High} cells which express high levels of the HCV entry factor CD81, compensating for their low permissiveness to HCV.^{26,27} IQGAP2 silencing significantly rescued HCV infection from IFN treatment with 10 IU/ml (Supplementary Figure 1A, 1B and 1C). These results indicate that IQGAP2's role in mediating IFN antiviral activity is not restricted to Huh7.5.1 cells used as a model in this study.

IQGAP2 is the only anti-HCV IEG in the IQGAP protein family

The IQGAP family proteins are expressed in the liver and are highly homologous.⁷ We investigated whether IQGAP1 and IQGAP3 were also involved in anti-HCV IFN responses. We verified the specificity and efficiency of siRNA silencing of IQGAP1, IQGAP2 or IQGAP3 in Huh7.5.1 (Supplementary Figure 2A). Neither IQGAP1 nor IQGAP3 silencing rescued HCV infection from IFN as measured by the percentage of infected cells (Supplementary Figure 2B), suggesting that the ability to mediate IFN antiviral response against HCV is specific to IQGAP2.

IQGAP2 is not involved in the classical JAK-STAT pathway

Antiviral ISGs are considered the primary effectors of the IFN antiviral response. We therefore evaluated whether IQGAP2 was induced by IFN. While Mx1 and OAS1, used as positive controls, were induced by IFN, we found that IQGAP2 expression level were not, (Supplementary Figure 3A) confirming that IQGAP2 is not an ISG.

Next we evaluated whether IQGAP2 plays a role in the activation of the JAK-STAT pathway by IFN. As expected, IFN treatment led to an increase in STAT1 phosphorylation in Tyr701 that was significantly inhibited after IFNAR silencing (Supplementary Figure 3B). Interestingly, IQGAP2 silencing had no effect on STAT1 activation by IFN in Huh7.5.1 cells (Supplementary Figure 3B). We further evaluated whether IQGAP2 was involved in ISRE promoter activity downstream of the JAK-STAT pathway using an IRSE promoter-dependent luciferase reporter. As expected, ISRE promoter activity was induced by IFN and significantly decreased after IFNAR silencing (Supplementary Figure 3C). However, following IQGAP2 downregulation, no change was observed in ISRE promoter activity (Supplementary Figure 3C), further suggesting that IQGAP2 does not act through the classical JAK-STAT pathway of IFN.

RelA mediates IFN anti-HCV activity

Using a proteomic approach in HEK-293T cells, Bouwmeester *et al.* identified IQGAP2 as a putative interactor with RelA, a subunit of the NF- κ B complex.²⁸ NF- κ B is well characterized to induce the expression of cytokines that mediate the inflammatory response after viral infection. Hence, we investigated whether RelA is involved in the IQGAP2 pathway of IFN antiviral response.

We first confirmed the interaction between RelA and IQGAP2 using a glutathione S-transferase (GST) pull-down. GST alone or RelA fused to GST (RelA-GST) and FLAG-tagged IQGAP2 (IQGAP2-FLAG) proteins were expressed in HEK-293T cells. GST-encoding proteins were purified using glutathione beads and the co-precipitation of IQGAP2-FLAG protein was assessed by Western blotting. IQGAP2-FLAG protein co-purified with the RelA-GST but not with GST alone (Supplementary Figure 4A). Reciprocally, endogenous RelA protein co-precipitated with IQGAP2 fused to GST (IQGAP2-GST) but not with the GST alone (Supplementary Figure 4B). These data confirm the interaction between IQGAP2 and RelA.

Next, we investigated whether RelA also played a role in the IFN antiviral response in Huh7.5.1 cells. RelA expression was significantly inhibited in Huh7.5.1 cells after siRNA transfection (Figure 2A). Interestingly, RelA knockdown mimicked IQGAP2 silencing and significantly rescued HCV infection from IFN treatment (Figure 2B, 2C and 2D) indicating that IQGAP2 and RelA cooperatively act to mediate the antiviral response of IFN.

IQGAP2 and RelA modulate permissiveness to HCV infection

We next investigated whether IQGAP2 and RelA exhibited antiviral activity in the absence of exogenous IFN administration. Consistent with a defective interferon production of Huh7.5.1 cells, none of the tested protein knock-downs, including IFNAR, affected HCV infection (Figure 3A, 3B and 3C). The absence of RelA effect is consistent with our previous reports that NF- κ B siRNA-mediated or chemical inhibition did not affect JFH1 replication in Huh7.5.1 cells.^{29,30} These results indicate that IQGAP2 and RelA require the presence of IFN to inhibit HCV infection in Huh7.5.1 cells, thus acting downstream the binding of IFN to its receptor. Interestingly, both IQGAP2 and RelA silencing enhanced HCV infection in

Huh7/CD81^{High} cells (Figure 3D, 3E and 3F), indicating that IQGAP2 and RelA modulate permissiveness to HCV in IFN-competent cells.

Together, these results suggest that both IQGAP2 and RelA modulate permissiveness to HCV in an interferon-dependent mechanism and act, at least in part, downstream of the binding of IFN to its receptor.

IFN activates the NF- κ B pathway in an IQGAP2-dependent manner

The finding that IQGAP2 and RelA, a component of the NF- κ B transcription factor, may act downstream of the binding of IFN to its receptor prompted us to investigate whether IFN treatment led to the activation of the NF- κ B pathway. We first assessed NF- κ B promoter activity using Huh7.5.1 cells stably transduced with a lentiviral vector encoding a GFP reporter under the control of a NF- κ B promoter (Huh7.5.1/pNF- κ B-GFP cells). We used TNF α as a positive control. Interestingly, treatment with 100 IU/ml IFN led to a significant increase in NF- κ B promoter activity in those cells (Figure 4A). We confirmed NF- κ B activation by IFN using two independent IFN batches (data not shown). Furthermore, NF- κ B was activated by IFN in a dose-dependent manner (Figure 4B) and IFNAR silencing prevented NF- κ B activation by IFN without affecting its basal activity (Figure 4C). Together, these data demonstrate that IFN treatment stimulates NF- κ B promoter in Huh7.5.1 cells.

We next determined whether IQGAP2 and RelA were necessary for NF- κ B promoter activation by IFN in Huh7.5.1/pNF- κ B-GFP cells. As expected, RelA silencing abrogated the basal activity of NF- κ B promoter and significantly limited its induction by IFN (Figure 4C). Similar to IFNAR silencing, IQGAP2 knock-down led to a significant decrease in NF- κ B promoter activation by IFN (Figure 4C) suggesting that NF- κ B promoter is stimulated by IFN involving IQGAP2 and RelA. Furthermore, the observation that IQGAP2 does not alter basal NF- κ B promoter activity suggests that the role of IQGAP2 in NF- κ B stimulation depends on the stimulus.

We then sought to determine if IQGAP2 played a role in RelA activation following IFN treatment in Huh 7.5.1 cells. RelA activation by phosphorylation in Ser536³¹ increased after IFN treatment and was significantly inhibited by IFNAR, IQGAP2 or RelA silencing (Figure 4D). A recent study showed that Iqgap2 knock-out mice had significantly less RelA in the liver compared to wild-type mice.³² In our study, the transient knockdown of IQGAP2 has no effect on NF- κ B basal activation (Figure 4C) or RelA expression (Figure 4D) allowing us to differentiate the roles of IQGAP2 and RelA. These data show that IFN activates RelA containing NF- κ B complex in an IQGAP2-dependent manner.

IQGAP2 and RelA are necessary for the expression of the complete anti-HCV ISG arsenal

As previously outlined, ISGs are the primary effectors of the IFN response. We thus investigated whether IQGAP2 and RelA played a role in the induction of antiviral ISGs by IFN. We selected 39 ISGs with previously described antiviral activity against HCV.^{33,34} We found that 23 of these 39 genes were induced at least 2-fold by IFN in Huh7.5.1 cells (Figure 5). IFNAR silencing was used as a positive control and resulted in significantly diminished expression of all 23 selected ISGs (Figure 5). Interestingly, both IQGAP2 and

RelA silencing significantly decreased the induction of the same 15 ISGs but did not affect the other 8 ISGs (Figure 5). These results demonstrate that IQGAP2 and RelA are necessary for the full induction of a subset of ISGs with anti-HCV properties.

As shown above, IQGAP2 and RelA are necessary for the NF- κ B promoter activation by IFN in Huh7.5.1 cells. We then assessed whether the subset of ISGs regulated by IQGAP2 and RelA were also NF- κ B target genes. The NF- κ B target gene resource (www.nf-kb.org) and the Champion ChIP Transcription Factor Search Portal based on the ENCODE database (www.sabiosciences.com) were used to identify genes harboring a NF- κ B binding site. Overall we found that 12 of the 23 selected genes were targeted by NF- κ B (RSAD2, Mx1, GBP1, ADAR, TRIM14, IRF1, PKR, STAT1, IRF2, B2M, RIG-I and CXCL11). Interestingly, all 12 genes were found to be co-regulated by IQGAP2 and RelA (Figure 5) suggesting that ISGs with an NF- κ B binding site are regulated by IQGAP2 and RelA. As for the 11 genes not previously described as NF- κ B targets, 8 ISGs were unchanged following IQGAP2 or RelA knockdown (IFITM1, ISG15, IFIT3, DDX60, PLSCR1, ISG12, IFITM3 and IFI44L) and 3 ISGs required IQGAP2 and RelA for their full induction by IFN (IFI6, OAS1 and IFIT1), probably resulting from a secondary response as the induction of transcription factors such as STAT1 and IRF2 were found to depend on IQGAP2 and RelA. Taken together, these data indicate that the IQGAP2-RelA pathway of IFN is necessary for the full induction of the anti-HCV ISG arsenal.

IQGAP2's role is independent of STAT1-mediated transcription of ISGs

To further investigate the relationship between the IQGAP2 and STAT1-mediated pathways, we compared the effect of IQGAP2 and STAT1 knock-downs on the kinetics of induction by IFN of four ISGs in Huh 7.5.1 cells. As expected, efficient siRNA-mediated STAT1 knock-down (Figure 6A) resulted in decreased induction of all four ISGs (Figure 6B). A similar pattern was observed after IQGAP2 silencing for RSAD2, Mx1 and GBP1, while ISG15 remained unchanged. Interestingly, STAT1 knock-down recapitulated IFNAR inhibition of ISG15 induction but was insufficient to completely block the induction of IQGAP2-dependent RSAD2 and GBP1, which was only observed with the additive effect of STAT1 and IQGAP2 double knock-down (Figure 6C), implying that IQGAP2 and STAT1 act in different pathways. These data show that the IQGAP2-RelA arm of IFN acts independently of the classical STAT pathway (Supplementary Figure 5).

Discussion

Our recent siRNA screen identified a large number of novel genes important for IFN-mediated anti-HCV responses,^{6,35} highlighting significant deficiencies in our understanding of antiviral responses. In the present study, we demonstrate a novel function of IQGAP2 in regulating the innate antiviral response, which seems independent of its role as a tumor-suppressor. We show that IQGAP2 mediates HCV infection inhibition by IFN independently of the classical STAT pathway (Supplementary Figure 5). We found that IQGAP2 requires the presence of IFN to mediate its anti-HCV response, suggesting a role downstream of IFN receptor engagement. We showed that IQGAP2, which physically interacts with the RelA subunit of the NF- κ B complex,²⁸ mediates the early activation of RelA by IFN and the

downstream activation of NF- κ B-dependent transcription. Finally, we showed that IQGAP2 and RelA are required for the full induction by IFN of a subset of ISGs with anti-HCV properties and previously described as NF- κ B-targets.

RelA is one of five proteins (with RelB, cRel, p50 and p52) that associate into homo- or heterodimers to form the NF- κ B transcription factors³⁸ that regulate the expression of hundreds of genes associated with diverse cellular processes, including antiviral responses. The participation of NF- κ B, and specifically RelA, in the antiviral response against HCV is not well understood. RelA was initially identified as a host factor supporting HCV subgenomic replicon replication in Huh7 and Huh7.5 cells.^{39,40} However, as observed in this study, NF- κ B or RelA inhibition demonstrated little to no effect on HCV JFH1 infection in IFN-defective Huh7.5.1 cells.^{29,30,41} We show that RelA silencing mimics IQGAP2 knock-down in rescuing HCV infection from IFN inhibition and in inducing ISGs in the absence of infection. These results indicate that RelA participates in the establishment of an antiviral state downstream of IFN binding to IFNAR, a novel mechanism separate from its well-characterized role in IFN production,⁴² observations supported by another report that IFN activates the RelA-containing NF- κ B transcription factor in the HepG2 hepatoma cell line.⁴³ NF- κ B activation by IFN and binding to DNA have also been described in a number of human and rodent cell lines including from renal, intestinal, lymphoblastoid and fibroblastic origins^{43–46}.

Given the pleiotropic nature of NF- κ B, the cellular responses downstream of RelA activation are thought to depend on the stimulus, the pathway of activation, the microenvironment and the cell type.³⁸ Consistently, NF- κ B activation by IFN has been shown to promote cell survival in human Daudi cells⁴³ whereas it down regulates IFN activity against vesicular stomatitis virus and influenza in mouse fibroblasts,^{45,47} possibly through the inhibition of the expression of a subset of ISGs.⁴⁵ In macrophages, a detailed examination of the mechanism of Nos2 promoter activation by *Listeria* revealed cooperation between the classical STAT and NF- κ B pathways remodeling ISG chromatin (Farlik M immunity 2010). In this model, PRR-activated NF- κ B binds to Nos2 promoter and recruits both histone acetyl transferase (HAT) and basal transcription factor TFIID associated with CDK7 kinase. In a second phase, the IFN-activated ISGF3 binds to the promoter and recruits both HATs and the polymerase II, which after activation by CDK7, initiates the transcription of Nos2 and a very limited subset of ISGs, suggesting the existence of different regulation mechanisms within the same model. Our data suggest that the IQGAP2-NF- κ B pathway may be sufficient to activate the transcription by IFN of a subset of ISGs, even in the absence of STAT1, consistent with the absence of interdependency between NF- κ B and ISGF3 in their promoter-binding and HAT-recruiting activities. However, the exact mechanism remains to be investigated.

The series of events leading to NF- κ B activation by IFN in an IQGAP2-dependent manner remain largely unknown. The IQGAPs scaffolds are thought to integrate and process signals via the modulation of the association with their binding partners, probably in response to conformational changes. (Elliott SF. World Journal of Biological Chemistry. 2012). Although not induced by IFN, IQGAP2 binding profile may be altered after posttranslational modifications. Indeed, IQGAP2 is a phosphoprotein with over 200 putative and one

confirmed phosphorylation site in Thr716, which was shown to modulate the strength of binding to Rac1 (Logue JS Journal of biological chemistry 2011). While little is known about IQGAP2 binding partners, the kinase Akt may be a potential mediator for NF- κ B activation by IFN. Indeed, Akt is an IQGAP1 interactor (Smith JM, Trends in cell biology 2015) and the high homology and similar structure between IQGAP1 and IQGAP2 supports the notion that Akt may also interact with IQGAP2. Moreover, Akt is activated by IFN in various models (Joshi A cytokine 2010), and has been shown to mediate NF- κ B activation by phosphorylation of the RelA subunit (Dan HC Genes Dev 2008). More studies are required to determine the dynamics of IQGAP2 binding profile and how this scaffold integrates signals downstream of IFN.

In conclusion, IQGAP2 has a novel role in mediating IFN antiviral responses by regulating ISGs in a NF- κ B-dependent manner. Given the pivotal role of ISGs in antiviral responses, IQGAP2 may also contribute to the control of other hepatotropic viruses. Moreover, the limited tropism of IQGAP2 expression, confined mostly to the liver, makes it an attractive target for future therapeutic development of more selective hepatoprotective NF- κ B modulators, particularly challenging given the ubiquitous and pleiotropic nature of NF- κ B. This warrants further evaluation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of Abbreviation

GST	Glutathione S-transferase
HAT	histone acetyltransferase
HCC	hepatocellular carcinoma
HCV	Hepatitis C virus
IEG	Interferon effector gene
IFN	Interferon
IFNAR	Interferon alpha receptor
IQGAP	IQ-motif containing GTPase activating protein
IRF	interferon regulatory factor
ISG	Interferon-stimulated gene
ISRE	Interferon-sensitive responsive element

JAK	Janus kinase
NF-κB	Nuclear factor κ B
PRR	Pattern Recognition Receptor
STAT	Signal transducer and activator of transcription
TNFα	Tumor necrosis factor alpha

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Figure 1. IQGAP2 silencing rescues HCV infection from IFN

(A) Huh7.5.1 cells were transfected with Non-targeting siRNA (NT) or IQGAP2 siRNA (#1 to 4 individually or pooled) and IQGAP2 silencing was evaluated 72 hours post-transfection (p.t) on total RNA extracts by qRT-PCR (Left) or on total protein extracts by Western Blotting (Right). (B) Huh7.5.1 cells were transfected with the indicated siRNA and cellular ATP levels were measured 4 days p.t. (C–E) Huh7.5.1 cells were transfected with the indicated siRNA, 2 or 3 days later, cells were treated with 100 IU/ml IFN for 24 hours and then infected with JFH1 at a MOI of 1 TCID₅₀/cell for an extra time as indicated or 48 hours. HCV infection was measured as (C) percentage of infected cells by fluorescence microscopy after HCV Core protein staining, (D) intracellular HCV RNA level in total RNA extracts by qRT-PCR and (E) infectious HCV particle production in the extracellular medium by TCID₅₀ assay.

Figure 2. RelA silencing rescues HCV infection from IFN

(A) Huh7.5.1 cells were transfected with the indicated siRNA and silencing was evaluated 72 hours p.t on total RNA extracts by qRT-PCR (Left) and on total protein extracts by Western Blotting (Right) (B–D) Huh7.5.1 cells were transfected with the indicated siRNA for 2 days, treated with IFN for 24 hours, infected with JFH1 at a MOI of 1 TCID₅₀/cell for an extra 48 hours and HCV infection was measured as (B) percentage of infected cells by fluorescence microscopy after HCV Core protein staining, (C) intracellular HCV RNA level in total RNA extracts by qRT-PCR and (D) infectious HCV particle production in the extracellular medium by TCID₅₀ assay.

Figure 3. IQGAP2 or ReIA silencing increases permissiveness to HCV in Huh7/CD81^{High} cells but not in Huh7.5.1 cells

(A–C) Huh7.5.1 cells or (D–F) Huh7/CD81^{High} cells were transfected with the indicated siRNA, infected 3 days later with JFH1 at a MOI of 0.5 TCID₅₀/cell for Huh7.5.1 cells or at a MOI of 1 TCID₅₀/cell for Huh7/CD81^{High}. Two days p.i., HCV infection was measured as (A,D) percentage of infected cells by fluorescence microscopy after HCV Core protein staining, (B,E) intracellular HCV RNA level in total RNA extracts by qRT-PCR and (C,F) infectious HCV particle production in the extracellular medium by TCID₅₀ assay.

Figure 4. IQGAP2 or RelA silencing inhibits NF-κB activation by IFN

(A) Huh7.5.1/pNF-κB-GFP cells were treated with 100 IU/ml IFN, mock-treated or treated with 10 ng/ml of TNFα for 48h and analyzed by fluorescence microscopy after nuclear DAPI staining. (B) Huh7.5.1/pNF-κB-GFP cells were treated for 48 hours with the indicated dose of IFN or 10 ng/ml TNFα or 10 μg/ml PBS-BSA corresponding to the concentration of PBS-BSA in IFN 1000 IU/ml solution (Vehicle) or mock-treated (Mock) and analyzed by Flow Cytometry. (C) Huh7.5.1/pNF-κB-GFP cells were transfected with the indicated siRNA, 72 h later, cells were treated with IFN or mock-treated for 48 h and analyzed by flow cytometry (Left) and representative fluorescent pictures are presented for the IFN treated cells after nuclear DAPI staining (Right). (D) Huh7.5.1 cells were transfected with the

indicated siRNA for 72 h, treated with IFN or mock-treated for 20 min. RelA activation by phosphorylation at Ser 536 was assessed on total protein extracts by Western blotting.

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Figure 5. IQGAP2 or RelA silencing decreases the induction by IFN of a subset of antiviral ISG
Huh7.5.1 cells were transfected with the indicated siRNA and treated 3 days later with IFN or Mock-treated for 8 hours. The indicated ISGs induction levels were measured on total RNA extracts by qRT-PCR. All values are normalized to Huh7.5.1 cells transfected with NT-siRNA and Mock-treated, set as 1. The dotted line separates previously reported NF-κB target genes on the left, from the genes unrelated to NF-κB on the right.

Figure 6. IQGAP2 and STAT1 have an additive effect on the induction by IFN of a subset of ISG
(A) Huh7.5.1 cells were transfected with the indicated siRNA and silencing was evaluated 72 hours p.t on total RNA extracts by qRT-PCR (Left) and on total protein extracts by Western Blotting (Right). **(B)** and **(C)** Huh7.5.1 cells were transfected with the indicated siRNA and treated 3 days later with IFN or Mock-treated. The indicated ISG induction levels were measured 8 hours p.t. unless stated otherwise on total RNA extracts by qRT-PCR. All values are normalized to Huh7.5.1 cells transfected with NT-siRNA (or double NT-siRNA) and Mock-treated, set as 1.