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Editorial

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Interferon revisited: Peering behind the lines of antiviral defense

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HCV is a positive-stranded RNA virus, that accounts for approximately 71 million chronically infected individuals worldwide and represents a major risk factor for liver fibrosis, cirrhosis and hepatocellular carcinoma.[1] For a long time, interferon (IFN)-alpha regimens were the cornerstone of HCV therapy, which depending on the HCV genotype, led to a sustained virologic response in 54–75% of patients.[2] However, therapy with IFNs is lengthy and burdensome for many patients due to the broad spectrum of adverse effects.[2] Thus, they have recently been replaced with more efficient and well-tolerated direct-acting antivirals, rendering chronic HCV infection a curable disease.[3] Over the last 30 years, HCV research has provided important insights into the molecular mechanisms of innate IFN responses, as well as the sophisticated viral strategies to evade the host defenses and to persist.[4] Viruses entering the host are detected by cellular sensors of pathogen-associated molecular patterns, leading to the production of type I and III IFNs. Subsequently, this triggers the rapid transcription of hundreds of IFN-stimulated genes (ISGs), which are directly or indirectly antiviral and control the IFN response itself.[5,6] The ISG C19orf66 is induced by several clinically relevant viruses including HCV, and thus may exhibit antiviral activity.[6] Indeed, it was previously described as a potent restriction factor for HIV, Kaposi's sarcoma-associated herpesvirus (KSHV), Zika virus and dengue virus (DENV).[7–10] However, the molecular details related to the function of C19orf66, especially for HCV, remained largely unknown.

In this regard, a new study published in this issue of *Journal of Hepatology* by Volker Kinast and co-workers, sheds new light on the role of C19orf66 as an IFN-induced restriction factor (Fig. 1).[11] Analyzing primary human hepatocytes infected with cell-culture-derived HCV (HCVcc) and liver biopsies from 25 patients with chronic HCV infection, the authors revealed significantly increased mRNA expression levels of *C19orf66*, that appeared to be largely independent of viral load, METAVIR score and HCV genotype in patients. Consistently, C19orf66 is induced by IFN therapy in patients with HCV, as revealed by computational analysis of liver transcriptomic data. Combination therapy of pegylated IFN-alpha (pegIFN-α) with ribavirin induced a peak of hepatic *C19orf66* mRNA expression at 4- and 16-hours post-treatment, highlighting it as an early-induced ISG. The authors demonstrated an antiviral effect on HCV using *C19orf66* knockout cell lines generated by CRISPR/Cas9. Disrupted C19orf66 expression restored IFN-α-suppressed replication of HCVcc and a subgenomic HCV replicon, further validating the antiviral effect of C19orf66 on HCV. While the impact of C19orf66 on other steps of the HCV life cycle, such as entry or translation, was not significant, the authors confirmed that C19orf66 is a restriction factor of HCV replication, using overexpression studies in combination with subgenomic replicons. The observed antiviral effect of C19orf66 seems independent from 7 tested HCV genotypes, suggesting an indirect "host targeting" impact of this ISG. This is further supported by the lack of an association of hepatic C19orf66 expression with the underlying HCV genotype in patients. C19orf66 seems to be recruited to lipid droplets in HCV-infected cells, where it partially colocalizes with the viral proteins core, NS3 and NS5A. In contrast, C19orf66 remains homogenously distributed in the cytosol of non-infected cells. These findings indicate that C19orf66 exerts its antiviral action at the HCV replication compartment of the membranous web (MW), which integrates lipid droplet accumulations as the central site of viral processing and particle formation.[12] The MW is formed after a massive remodeling of membranes from the endoplasmic reticulum (ER),[12] which involves a HCV-induced stimulation of phosphatidylinositol 4-kinase (PI(4)K). This leads to an enrichment of phosphatidylinositol 4-phosphate (PI(4)P) at the membranes of the ER,[13] thus provoking a bending and deformation of double-stranded ER membranes in HCV-infected cells. Interestingly, the authors established a functional link between C19orf66 expression and impaired HCV-induced PI(4)P levels in HCV replicating cells. Moreover, expression of C19orf66 with mutated zinc-finger motif (C19orf66-Zinc^{mut}) impaired its antiviral activity, coinciding with a less perturbed MW morphology and composition compared to cells expressing wild-type C19orf66.

In addition to the identified antiviral role of C19orf66 on MW formation, the authors identified that stress granule-associated nucleoproteins RO60, RBPMS and CELF1 interacted with C19orf66. Since this association required the zinc-finger motif of C19orf66, the authors suggested a role of this ISG in stress granule formation with functional relevance for its antiviral

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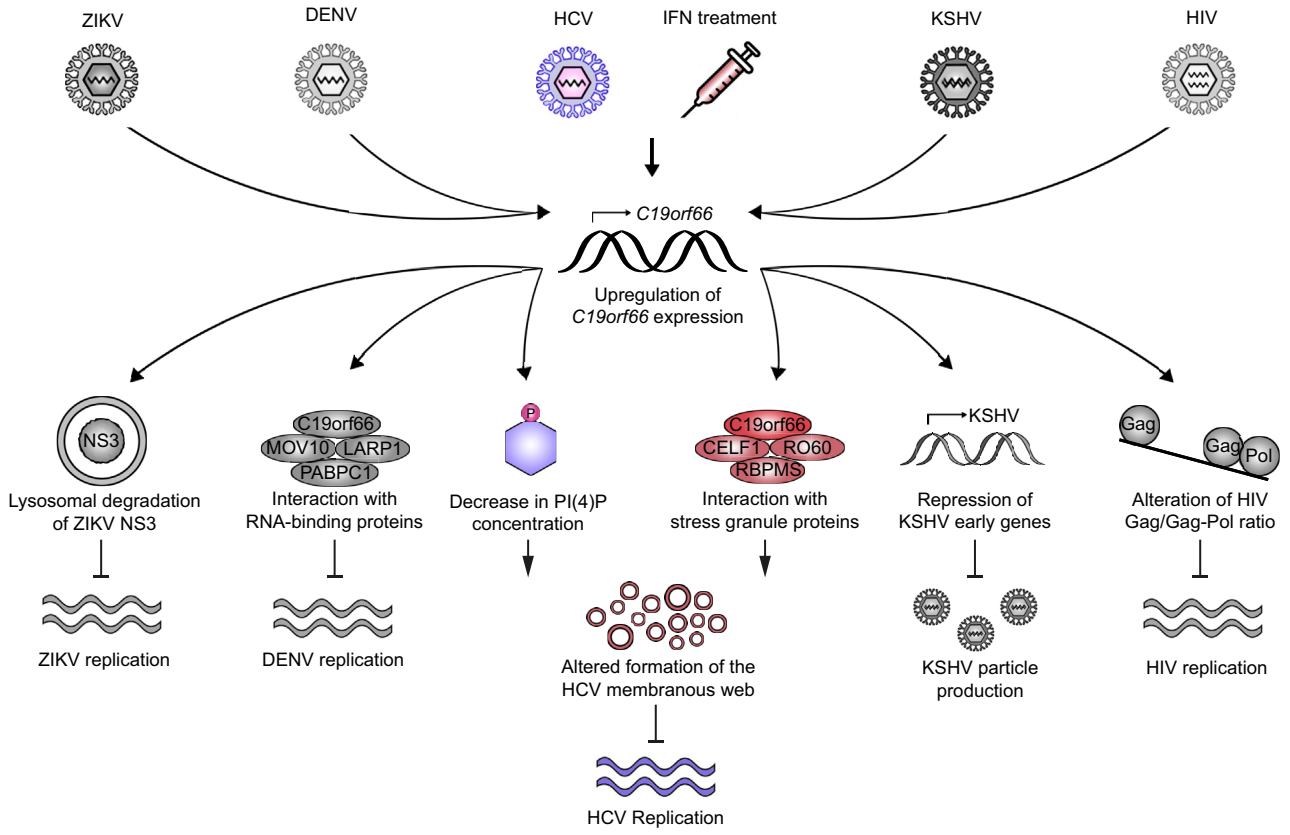

Fig. 1. C19orf66 is a broadly acting ISG that exhibits a pluripotent and mechanistically diverse antiviral activity on clinically relevant viruses. C19orf66 upregulation has been previously shown to inhibit viral replication via lysosomal degradation of NS3 in the case of ZIKV, by interaction with RNA-binding proteins in the context of DENV infection and through alteration of the Gag/Gag-Pol ratio in the course of HIV life cycle. Additionally, C19orf66 was reported to repress the expression of KSHV early genes, having as a consequence an impaired viral particle production. Volker Kinast and co-workers demonstrate that upregulation of C19orf66 in the context of HCV infection or IFN treatment impairs the HCV cycle specifically at the replication step. The mechanism behind this antiviral effect implicates the altered formation of the HCV MW, originating from the interaction of C19orf66 with stress granule proteins and the downregulation of PI(4)P levels. DENV, dengue virus; IFN, interferon; ISG, IFN-stimulated gene; KSHV, Kaposi's sarcoma-associated herpesvirus; MW, membranous web; PI(4)P, phosphatidylinositol 4-phosphate; ZIKV, Zika virus.

function. This is supported by previous studies on DENV, where infection provoked cytoplasmic ribonucleic C19orf66-containing granule formation, while granule disruption partially rescued viral replication.[7,14] The findings of Volker Kinast and co-workers once more highlight the pluripotent character of the complex IFN response against a pathogen.[5,6] Similar to adaptive immunity, where random pre-existing immunoglobulins react to a novel immunogen and thus lead to the clonal expansion of a pathogen-specific antibody, ISGs are able to target a large variety of host processes that are relevant to previously encountered pathogens, and may be relevant to future pathogens.

C19orf66 is thus another example of how evolution created ISGs as a universal tool set. Like a swiss army knife, ISGs can act with various blades of the same tool against different pathogens. While many putative functions of C19orf66 may not be relevant to HCV infection, this protein certainly inhibits other viruses with different aspects of its pluripotent nature (Fig. 1), *i.e.*, triggering the lysosomal degradation of ZIKA NS3,[8] repressing KSHV gene expression,[9] altering crucial Gag/Pol ratios during HIV replication,[10] stress granule formation during DENV[7] and HCV infection, and most likely additional not yet discovered facets of its action relevant to other pathogens. Interestingly, C19orf66 is also induced in the antiviral response to SARS-CoV,[9] where it escapes the virus-induced mRNA degradation, as has been demonstrated for KSHV.[9] However, whether C19orf66 has antiviral actions against coronavirus infections remains unclear. Evolution shaped the IFN response as a powerful innate defense mechanism for the eradication of invading pathogens. Understanding the mechanisms of this cellular toolset, as well as the evasion strategies of certain viruses such as HCV, gives important clues on their Achilles' heels and thus may also pave the way to understand and to tackle future emerging viral diseases.

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Conflict of interest

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

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Authors' contributions

All authors conceived, wrote, and reviewed the manuscript.

Supplementary data

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Author names in bold designate shared co-first authorship

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