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Revisiting the Paradox of ISG expression as a predictor of HCV treatment response, a decade later

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Endogenous interferon signaling is the body's first line of defense against viruses, providing the rationale for use of IFN as the backbone of treatment for viral hepatitis for over two decades. Hepatitis C viral RNA is recognized by innate pattern recognition receptors (PRRs) residing within the cytoplasmic or endosomal compartments. These PRRs include the cytoplasmic sensor retinoic acid inducible gene-I (RIG-I), Nod-like receptors, and endosomal toll-like receptor-3 (TLR3). Activation of PRRs by HCV leads to IFN regulatory factor 3 (IRF3) phosphorylation, dimerization and nuclear translocation inducing transcription of type I and type III interferon (IFN) genes. Binding of IFNs to their cognate receptor activates the JAK/STAT pathway, leading to the formation of Stat1/2/IRF9 complexes that induce expression of interferon stimulated genes (ISGs), key players in the innate immune response to viral infection. The IFN/ISG response is critically involved in the suppression of HCV replication. Despite robust upregulation of IFNs and ISGs in the liver (1, 2), spontaneous clearance of HCV occurs in less than 20% of infected individuals. The ability of HCV to establish chronic infection can in part be attributed to several strategies HCV has evolved to antagonize the anti-viral actions of IFNs and ISGs, e.g., HCV NS3/4A protease cleaves essential signaling adapter molecules mitochondrial antiviral signaling (MAVS, also known as CARDIF/IPS-1/VISA) and TRIF, thus preventing downstream activation of IRF3 (3). Once sufficient viral proteins have accumulated in the cytosol, HCV uses its multifunctional NS3/4A protease, essential for HCV replication, to cleave MAVS and ablate RIG-I mediated innate immune signaling (4). Moreover, not all ISGs benefit the host and several ISGs (including ISG15 and USP-18) have been reported to have pro-viral properties (5).

In 2008, Sarasin-Filipowicz and colleagues reported the somewhat counterintuitive finding (2) that chronically HCV-infected patients non-responsive to IFN-based antiviral therapy demonstrated higher baseline hepatic ISG expression, evidence of more active innate immune response, and refractoriness to further IFN stimulation. In that seminal study, paired biopsies were analyzed pre-treatment and 4 hours after first dosing of PEG-IFN-α, the time point being selected based on chimpanzee data showing maximal induction of ISG

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expression. Patients with rapid response to IFN-based therapy showed strong up-regulation of ISGs, whereas those who failed therapy showed pre-activation of their hepatic IFN system without a significant treatment-associated induction. These latter patients with robust ISG expression might have been predicted to spontaneously resolve HCV infection and yet developed persistent infection and were non-responsive to IFN-based therapy. Moreover, there was not a significant difference in the expression of negative regulators of IFN signaling (e.g., SOCS1, SOCS3, USP18) in the PEG-IFN-α-stimulated liver biopsies of responders versus non-responders. It is very unlikely that a single ISG is the "key anti-HCV ISG" that determines viral clearance either during natural infection or in response to IFN-based therapy; but rather, it is likely the aggregate action of these ISGs, in concert with the adaptive immune response, that govern the outcome of the infection. Subsequent studies confirmed the inverse correlation with hepatic ISG set point levels and outcome of IFN therapy and showed cell-type specific differences in ISG expression (1). Induction of Type III IFNs is the predominant antiviral pathway and driver of ISG induction which render hepatocytes refractory to further type I IFN action (6), conceptually supported by the observation that blocking Type III IFN enhances the antiviral activity of exogenous IFN-α (5).

Interestingly, in the current issue of *Hepatology*, Ghany and colleagues from the National Institutes of Health (ref 7) demonstrate that hepatic ISG expression is a favorable predictor of response to direct-acting antivirals (DAAs) and that transcription of ISGs is downregulated rapidly (within 4 weeks) following initiation of DAAs. All patients were genotype 1b-infected, had failed therapy with PEG-IFN-α/Ribavirin, and were treated with asunaprevir (NS3 protease inhibitor) and daclatasvir (an NS5A inhibitor) for 24 weeks. Among the 11 patients in the longitudinal transcriptome analysis, seven achieved undetectable HCV RNA 12 weeks post-therapy (SVR_{12}), and four experience virological breakthrough between treatment weeks 4 and 12. Pre-treatment ISGs with the most robust expression included CXCL10, CXCL11, ISG15, CCL20, DDX60, RSAD2 (viperin), MX1, IFIT1, SOCS1, and USP18. The inclusion of a protease inhibitor (PI) in the dual therapy inhibits replication but might also be expected to rescue MAVS/RIG-I signaling at an earlier time point than regimens not including PIs that suppress replication, preventing the synthesis of new HCV protease but not affecting NS3/4A present in the cells prior to treatment. Accordingly, it would have been interesting to determine early viral decline in the responders versus those with virological breakthroughs in the current cohort. It is tempting to consider whether higher hepatic ISGs at baseline might lead to accelerated viral kinetics (and correction of immune dysregulation), identifying patients in whom a shorter course of DAA treatment might be considered without compromising SVR_{12}. In the context of fewer liver biopsies being performed in clinical practice and the absence of a peripheral surrogate for hepatic signature, it might not be a pragmatic issue. It would also be interesting to determine if the pre-treatment transcriptional signatures shown in this study would still be predictive of viral response in patients receiving optimal DAA combinations with expected SVR rates exceeding 95%. In the current study, on-treatment time points demonstrated greater reduction of ISG expression in patients with SVR_{12} compared to those with virological breakthrough. A prior study by Meissner et al. comparing hepatic gene expression before and 24 weeks after treatment with sofosbuvir/ribavirin treatment showed

downregulation of a panel of ISGs, including members of the ISGF3 complex (STAT1, STAT2, and IRF9) and those associated with anti-HCV activity (IRF1, IFIH1, IRF7, PLSCR1, IFIT3, TRIM14, and IFITM1) (8). Moreover, a recent study by our group (9) using HCV genotype 1a-infected human liver-chimeric FRG mice (presumably lacking human nonparenchymal cells) and treated with daclatasvir, asunaprevir, and sofosbuvir demonstrated downregulation of ISGs (including CXCL10 and CXCL11) within 14 days. This was accompanied by increased protein expression of MAVS and an increase in the protein IFITM1, a tight junction protein only induced by IFN, that inhibits HCV entry; thus, DAAs may additionally block HCV spread among hepatocytes despite a decrease in ISGs.

In order to more comprehensively examine the role of innate immunity in DAA-mediated clearance, the current study from the NIH also includes analysis of peripheral natural killer (NK) cells, which represent the primary IFN-sensitive innate immune effector cell type, as biomarkers of peripheral IFN responses. Their results suggest that an increased NK response to IFN at baseline predicts DAA-induced SVR and that NK cell activity early on treatment distinguishes responder from viral breakthrough patients. This finding is in agreement with our own study of patients treated with DAAs (ledipasvir/sofosbuvir) which demonstrated early activation of NK cells suggesting a role for innate immunity in successful IFN-free regimens (10).

In summary, the authors should be commended for providing novel insights into the decade-old paradigm of ISG expression as a predictor of HCV treatment outcome.

References:

1. Chen L, Borozan I, Sun J, Guindi M, Fischer S, Feld J, Anand N, et al. Cell-type specific gene expression signature in liver underlies response to interferon therapy in chronic hepatitis C infection. Gastroenterology 2010;138:1123–1133 e1121–1123. [PubMed: 19900446]
2. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci U S A 2008;105:7034–7039. [PubMed: 18467494]
3. Rosen HR. Emerging concepts in immunity to hepatitis C virus infection. J Clin Invest 2013;123:4121–4130. [PubMed: 24084744]
4. Horner SM, Gale M Jr. Regulation of hepatic innate immunity by hepatitis C virus. Nat Med 2013;19:879–888. [PubMed: 23836238]
5. Sung PS, Hong SH, Chung JH, Kim S, Park SH, Kim HM, Yoon SK, et al. IFN-lambda4 potently blocks IFN-alpha signalling by ISG15 and USP18 in hepatitis C virus infection. Sci Rep 2017;7:3821. [PubMed: 28630501]
6. Thomas E, Gonzalez VD, Li Q, Modi AA, Chen W, Noureddin M, Rotman Y, et al. HCV infection induces a unique hepatic innate immune response associated with robust production of type III interferons. Gastroenterology 2012;142:978–988. [PubMed: 22248663]
7. reference for Ghany paper
8. Meissner EG, Wu D, Osinusi A, Bon D, Virtaneva K, Sturdevant D, Porcella S, et al. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. J Clin Invest 2014;124:3352–3363. [PubMed: 24983321]
9. Burchill MA, Roby JA, Crochet N, Wind-Rotolo M, Stone AE, Edwards MG, Dran RJ, et al. Rapid reversal of innate immune dysregulation in blood of patients and livers of humanized mice with HCV following DAA therapy. PLoS One 2017;12:e0186213. [PubMed: 29040318]

10. Golden-Mason L, McMahan RH, Kriss MS, Kilgore AL, Cheng L, Dran RJ, Wieland A, et al. Early and late changes in natural killer cells in response to ledipasvir/sofosbuvir treatment. Hepatol Commun 2018;2:364–37. [PubMed: 29619416]