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ADAR1 affects HCV infection by modulating innate immune response

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ABSTRACT

The hepatitis C virus (HCV) is a globally prevalent infectious pathogen. As many as 80% of people infected with HCV do not control the virus and develop a chronic infection. Response to interferon (IFN) therapy is widely variable in chronic HCV infected patients, suggesting that HCV has evolved mechanisms to suppress and evade innate immunity responsible for its control and elimination. Adenosine deaminase acting on RNA 1 (ADAR1) is a relevant factor in the regulation of the innate immune response. The loss of ADAR1 RNA-editing activity and the resulting loss of inosine bases in RNA are critical in producing aberrant RLR-mediated innate immune response, mediated by RNA sensors MDA5 and RIG-I. Here, we describe ADAR1 role as a regulator of innate and antiviral immune function in HCV infection, both *in vitro* and in patients. Polymorphisms within *ADAR1* gene were found significantly associated to poor clinical outcome to HCV therapy and advanced liver fibrosis in a cohort of HCV and HIV-1 coinfected patients. Moreover, ADAR1 knockdown in primary macrophages and Huh7 hepatoma cells enhanced IFN and IFN stimulated gene expression and increased HCV replication *in vitro*. Overall, our results demonstrate that ADAR1 regulates innate immune signaling and is an important contributor to the outcome of the HCV virus–host interaction. ADAR1 is a potential target to boost antiviral immune response in HCV infection.

1. Introduction

Hepatitis C virus (HCV) is a major public health problem that infects approximately 70 million people worldwide (Lavanchy, 2009). HCV infections become chronic in the majority of infected individuals and is a major cause of liver disease and hepatocellular carcinoma (Lauer and Walker, 2001). HCV infection occurs within hepatocytes and thus, the first line of immune defense against HCV relies on cell-intrinsic innate immunity within hepatocytes (Heim, 2013).

HCV is recognized by RIG-I (Loo et al., 2006), TLR3 (Seki and Brenner, 2008; Wang et al., 2009) and PKR (Arnaud et al., 2011) within hours of infection and activates the downstream signaling before extensive viral protein synthesis (reviewed in (Horner and Gale, 2013). However, despite the effective detection of viral infection, as many as 80% of people infected with HCV do not effectively control the virus and develop a chronic infection (Seeff, 2009), reflecting the fact that HCV has evolved several mechanism to evade and suppress innate immunity (reviewed in (Horner and Gale, 2009). Due to the crucial role of innate immune activation and the interferon pathway in achieving HCV viral clearance, it is not surprising that genes involved in this pathway may alter a patient's outcome of HCV infection. Genetic polymorphisms in, as interleukin-28B gene (IL-28B, also referred as IFN-λ3) (Ge et al., 2009; Rauch et al., 2010; Suppiah et al., 2009; Tanaka et al., 2009) (Rauch et al., 2010; Thomas et al., 2009) (Aparicio et al., 2010)*IFNL4* (Bibert et al., 2013; Franco et al., 2014; Prokunina-Olsson et al., 2013) and other genes in the IFN-alpha pathway have been associated to responses to antiviral therapy of chronic HCV infection (Medrano et al., 2017; Welzel et al., 2009).

IFN expression drives the expression of hundreds of ISG, encoding innate immune effectors that also exert control on virus replication and spread. For HCV, this set of genes includes distinct IRF family members, signal transduction factors and other ISGs of unknown mechanism of

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action, such as ADAR1 (Reviewed in (Horner and Gale, 2013)). Adenosine deaminases acting on RNA (ADAR) catalyze the conversion of adenosine (A) to inosine (I) in double-stranded RNA (dsRNA) substrates, a process of broad physiologic importance (George et al., 2014; Nishikura, 2016). Three ADAR enzymes (ADAR1-3) are present in humans, albeit ADAR1 has been shown to play more significant roles in biological and pathological conditions, including infection, autoimmune disease and cancer (Song et al., 2016). Of note, ADAR1 has been identified as a relevant factor in the regulation of the innate immune response [reviewed in (Wang et al., 2017)], suggesting that without appropriate RNA editing by ADAR1, endogenous or exogenous RNA transcripts may stimulate cytosolic RNA sensing receptors and therefore activate the IFN-inducing signaling pathways (Liddicoat et al., 2015). Although how RNA sensors differentiate “self” from “non-self” nucleic acids, such as double-stranded RNA (dsRNA), is not completely understood, accumulating evidences point towards ADAR1 as the crucial player; editing of RNA by ADAR1 differentiates “self” RNA from “non-self” RNA, leading to the detection of pathogenic material by the cytosolic RNA surveillance machineries (RIG-I)-like receptors (RLRs), including melanoma differentiation-associated protein 5 (MDA5) and RIG-I (Chung et al., 2018; George et al., 2016; Mannion et al., 2014). Both these RLRs interact with the mitochondrial activation signaling (MAVS) protein, ultimately activating transcription factors that initiate the expression of immune response genes, ranging from interferon (IFN) to antiviral genes (Brubaker et al., 2015; Tamura et al., 2008).

Growing evidences support a role for A-to-I editing in dsRNA by ADAR1 in the replication process of different viruses, but ADAR1 function has been associated either to enhanced or reduced virus growth or persistence (Samuel, 2011). In the case of HCV, ADAR1 has been shown to play a role in limiting HCV replication, by specifically eliminating HCV RNA through A-to-I editing (Taylor et al., 2005). Additionally, ADAR1 have also been associated to an essential role in hepatic homeostasis in knockout mice, while the loss of ADAR1 in hepatocytes has been associated to activated IFN signaling pathways, inflammation, injury, disruption of liver's integrity, fibrosis and extensive liver damage (Wang et al., 2015). However, activation of the endogenous IFN system may not only be ineffective in clearing HCV infection but also may impede the response to IFN therapy, most likely by inducing a refractory state of the IFN signaling pathway as previously indicated (Sarasin-Filipowicz et al., 2008). The exact mechanism underlying the antiviral role of ADAR1 in HCV infection is not clear and it deserves further investigation.

Here, we have investigated the role of ADAR1 as a regulator of innate and antiviral immune function in HCV infection, both *in vitro* and in HCV and HIV coinfected patients. Polymorphisms within *ADAR1* gene were found significantly associated to poor clinical outcome to HCV therapy and advanced liver fibrosis in coinfected patients. Moreover, we show that depletion of ADAR1 modifies IFNbeta and ISG expression and leads to increased HCV replication *in vitro*. Overall, our results demonstrate that ADAR1 is an important contributor to the outcome of the HCV virus–host interaction and point to ADAR1 as a potential target to boost antiviral immune response.

2. Materials and methods

2.1. Patients and samples

A cohort of 155 patients from our clinical unit with chronic HCV/HIV-1 coinfection who had a standard course of treatment with PEGIFNα/Ribavirin (RBV) with known virological response status at 24 weeks post-therapy were included in this study (Table 1) (Table 2). Treatment success (i. e. sustained-IFN viral response, SVR) was defined as undetectable HCV RNA in plasma 24 weeks after finishing therapy. Severe fibrosis was considered in patients with a METAVIR score ≥ F3. HCV viral load, HCV genotype, HIV-1 viral load, $CD4^+$ T cell count and transaminases levels were determined with conventional methods as described before (Aparicio et al., 2010).

The protocol was approved by the scientific committee of Hospital Universitari Germans Trias I Pujol. Written informed consent was obtained from each patient who participated in the study. All methods were carried out in accordance with relevant guidelines and regulations and to the ethical principles suggested in the Declaration of Helsinki.

2.2. DNA extraction and SNP genotyping

Blood samples from the patients were collected into EDTA tubes following standard procedures. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the QuickExtract™ DNA Extraction Solution 1.0 (EPICENTRE Biotechnologies). Briefly, 1×10^6 cells were resuspended in 0.5 ml of QuickExtract DNA Solution, incubated at 65 °C for 15 min, followed by an incubation of 2 min at 98 °C, and then stored at −30 °C.

2 μl of the extracted DNA were used for the genotyping. Two ADAR1 SNPs (rs2229857 and rs1127326, Human Genome hg38 assembly) were selected based on previous functional evidences (Medrano et al., 2017). The variants were typed using TaqMan SNP genotyping assay (Assay num: C_1235929_10 and C_8724402_20, respectively, Applied Biosystems) following manufacturer's protocol. Reactions were analyzed on an ABI PRISM 7500 (Applied Biosystems). Allele calling was performed using AutoCaller Software v 1.1 (Applied Biosystems). Genotyping data, minor allele frequency and Hardy-Weinberg equilibrium are found in Supplementary Table 1.

2.3. Statistical analysis

Treatment baseline covariates were analysed with chi-squared test, unpaired *t*-test and the Mann-Whitney *U* test (GraphPad Prism, version 4.00; GraphPad Software Inc., San Diego, California, USA). The association of each SNP and odds ratio and CI were computed using a stratified analysis by gender with a logistic regression model adjusted by HCV viral load and *IL28*B-rs12979860 and HCV genotype (Gonzalez et al., 2007) (Gonzalez et al., 2007). We consider four inheritance models (codominant, dominant, recessive model, and log-additive), and akaike information criterion (AIC) was considered for estimation of the relative quality of statistical models. P values were derived from likelihood ratio tests. In all cases, the reference class was set as the homozygosis for the major allele among controls. The level of significance was set up equal to 0.0083 (0.05/6 = 0.0083) based on Bonferroni correction for multiple comparisons. We considered gender and the two considered outcomes (response to treatment and fibrosis). Since two assessed variants are highly correlated (r2 = 0.99), Bonferroni correction for two variants was considered too conservative and not considered (Gao et al., 2008). All analyses were carried out using the SNPassoc R library (Gonzalez et al., 2007).

Experimental data are presented as mean ± SD. Paired Student's *t*-test was used for comparison between two groups, using the GraphPad Prism software. p-values lower than 0.05 were considered significant.

2.4. Cells

PBMC were obtained from blood of healthy donors using a Ficoll-Paque density gradient centrifugation and monocytes were purified using negative selection antibody cocktails (StemCell Technologies) as described before (Badia et al., 2016). In brief, monocytes were cultured in complete culture medium (RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) and penicillin/streptomycin (Gibco) and differentiated to monocyte derived macrophages (MDM) for 4 days in the presence of monocyte-colony stimulating factor (M-CSF, Peprotech) at 100 ng/ml. The protocol was approved by the scientific committee of Institut de Recerca de la Sida - IrsiCaixa. Buffy coats were purchased from the Catalan Banc de Sang i Teixits (http://www.bancsang.net/en/index.html). The buffy coats

Table 1
Clinical characteristics of 155 HCV/HIV-1 coinfected patients with known response to the IFN/RBV therapy.

	Sustained-IFN viral response	Non-Sustained IFN viral response	p-value
Patients, n (%)	60 (38,7)	95 (61,3)	–
Age, yr (median, IQR)	47,5 (45–52)	47 (44–49,5)	0.1245
Gender, n (%)			
Female	20 (33,3)	27 (28,4)	
Male	40 (66,6)	68 (71,6)	0.52
CD4 Absolute (cells/mm3) (mean ± SEM)	587,6 ± 32,6	589,4 ± 27,34	0.97
HCV genotype, n (%)			
1	25 (41,6)	59 (62,1)	
3	27 (45)	18 (18,9)	
4	8 (13,3)	18 (18,9)	**0.0023**
Fibrosis stage, n (%)			
F0-F2	36 (60)	59 (62.1)	
F3-F4	18 (30)	32 (33.7)	
Unknown	6 (10)	4 (4.2)	0.82
ALT (U/L) (mean ± SEM)	90.68 ± 11.16	89.43 ± 5.052	0.9088
AST (U/L) (mean ± SEM)	60.48 ± 6.688	66.09 ± 3.406	0.4121
Baseline HCV RNA Viral load (IU/ml) log10 (median,IQR)	5,84 (5,48-6,02)	5,93 (5,7-6,33)	**0.0097**
Undetectable HIV-1, n (%)	46 (76,6)	79 (83,1)	0.32
On Antiretroviral treatment, n (%)	58 (96,6)	93 (97,9)	0.64

$CD4^+$, ALT, AST, unpaired *t*-test; BL HCV VL, age, Mann-Whitney; HCV genotype, fibrosis, Gender, undetectable HIV-1, on ART, Chi-square test. Graph Pad Prism 4.0.

received were totally anonymous and untraceable and the only information given was whether or not they have been tested for disease. All donors provided informed consent at the time of blood extraction.

MT4, Jurkat and ACH2 cells (AIDS Reagent Program, National Institutes of Health, NIH, Bethesda, MD) were grown in RPMI 1640 L-Glutamine medium (Gibco, Madrid, Spain), supplemented with 10% heat-inactivated fetal calf serum (FCS; Lonza, Barcelona, Spain). The Hela-derived cell line TZM-bl (AIDS Reagent Program, National Institutes of Health, NIH, Bethesda, MD), were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Madrid, Spain), supplemented with 10% heat-inactivated fetal calf serum (Gibco, Madrid, Spain). The hepatoma cell line Huh7 cells and its derivative Huh7.5 expressing RFP-NLS-IPS and a constitutive mitochondrial marker, mito-EGFP (Jones et al., 2010) was a king gift of Dr. Charles M. Rice (The Rockefeller University). Both hepatoma cell lines were cultured DMEM with 10% FCS (Hyclone, Cultek) and supplemented with glucose (4.5 g/l; Invitrogen), 2 mM L-Glutamine (Sigma-Aldrich), 1 mM sodium pyruvate (Sigma-Aldrich) and 1X MEM Non-essential Amino Acid (M7145, 100× MEM Non-essential Amino Acid Solution NEEA, Sigma-Aldrich).

2.5. RNA interference

Isolated monocytes were transfected as previously described (Badia et al., 2016). Briefly, 50 pmol of the corresponding siRNA (siGENOME SMARTpool from Dharmacon, Thermo-Scientific, Waltham, USA and ThermoFisher Scientific), were transfected using a Monocyte Amaxa Nucleofection kit (Lonza, Basel, Switzerland) following manufacturer instructions. Monocytes were left untreated overnight and then differentiated to macrophages as described above. For transfecting Huh7 and Huh7.5 cells, the corresponding siRNAs were mixed with Lipofectamine 2000 reagent (Invitrogen) at a final concentration of 100 nM and let stand for 20 min. Then, lipofectamine-siRNA complexes were mixed with 2×10^5 cells and seeded in 24-well plates in the absence of serum, using OPTIMEM medium (Invitrogen) as described (Ballana et al., 2010). After 24 h, medium with serum was added and left untreated 24 h more. 48 h after transfection cells were detached using an EDTA solution (Versene, ThermoFischer Scientific), counted and seeded at appropriate concentration for viral infection. At the same time, knockdown phenotype was assessed by measuring RNA expression (1×10^5 cells were used for RNA extraction) and protein expression (8×10^5 cells were used for protein lysates).

A non-targeting siRNA (siNT, ON-TARGETplus Non-targeting Pool,

Table 2
Clinical characteristics of HCV/HIV-1 coinfected patients with different liver fibrosis stages (n = 147).

	Non-cirrhosis (F0 to F3)	Cirrhosis (F4)	p-value
Patients, n (%)	130 (88.5)	17 (11.6)	–
Age, yr (median, IQR)	47 (44.5–50)	47 (44.5–53)	0.7284
Gender, n (%)			
Female	38 (29.2)	6 (35.3)	
Male	92 (70.7)	11 (64.7)	0.6077
$CD4^+$ Absolute (cells/mm3) (mean ± SEM)	588.4 ± 22.57	570.7 ± 71.66	0.7931
HCV genotype, n (%)			
1	74 (56.9)	7 (41.2)	
3	35 (26.9)	6 (35.3)	
4	21 (16.1)	4 (23.5)	0.4641
ALT (U/L) (mean ± SEM)	87.56 ± 5.87	103.9 ± 14	0.3378
AST (U/L) (mean ± SEM)	61.78 ± 3.459	82.18 ± 13.39	0.0586
Baseline HCV RNA Viral load log10 (IU/ml) (median, IQR)	5.94 (5.7–6.10)	5.8 (5.55–6.6)	0.9050
Undetectable HIV-1, n (%)	103 (79.2)	15 (88.2)	0.58
On Antiretroviral treatment, n (%)	128 (98.4)	17 (100)	0.61

$CD4^+$, ALT, AST, unpaired *t*-test; Baseline HCV RNA Viral Load, Age, Mann-Whitney; HCV genotype, Gender, undetectable HIV-1, on ART, Chi-square test (GraphPad Prism 4.0). Eight patients without liver fibrosis data were excluded from the analysis.

Table 3
Association of ADAR1 rs2229857 SNP with Non-Sustained IFN viral response in HCV/HIV-1 coinfected patients treated with IFN plus Ribavirin.

Gene	SNP	Gender	Genetic model	Non-Sustained IFN viral Response	%	Sustained IFN Viral Response	%	OR	95%CI	p-value	AIC	p-value2
		All (n = 150)	**Codominant**									
			C/C	44	47,3	27	47,4	1		0,26	181,4	0,1
			T/C	31	33,3	25	43,9	1,5	0.67–3.38			
			T/T	18	19,4	5	8,8	0,56	0.16–1.93			
			Dominant									
			C/C	44	47,3	27	47,4	1		0,67	181,9	0,83
			T/C-T/T	49	52,7	30	52,6	1,17	0.55–2.48			
			Recessive									
			C/C-T/C	75	80,6	52	91,2	1		0,19	180,4	0,06
			T/T	18	19,4	5	8,8	0,47	0.14–1.53			
			log-Additive									
			0,1,2	93	62	57	38	0,92	0.54–1.55	0,74	181,9	0,45
ADAR1	rs2229857	Male (n = 104)	**Codominant**									
			C/C	29	43,9	19	50	1		**0,021**	121,7	**0.0031***
			T/C	21	31,8	18	47,4	1,48	0.55–3.96			
			T/T	16	24,2	1	2,6	0,11	0.01–1.02			
			Dominant									
			C/C	29	43,9	19	50	1		0,83	127,4	0,84
			T/C-T/T	37	56,1	19	50	0,9	0.36–2.26			
			Recessive									
			C/C-T/C	50	75,8	37	97,4	1		**0.007***	120,3	**0.0012***
			T/T	16	24,2	1	2,6	0,09	0.01–0.83			
			log-Additive									
			0,1,2	66	63,5	38	36,5	0,63	0.32–1.25	0,18	125,6	0,1
		Female (n = 46)	**Codominant**									
			C/C	15	55,6	8	42,1	1		0,35	62,6	0,43
			T/C	10	37	7	36,8	1,68	0.37–7.58			
			T/T	2	7,4	4	21,1	5,51	0.43–71.16			
			Dominant									
			C/C	15	55,6	8	42,1	1		0,27	61,5	0,47
			T/C-T/T	12	44,4	11	57,9	2,17	0.53–8.91			
			Recessive									
			C/C-T/C	25	92,6	15	78,9	1		0,2	61,1	0,2
			T/T	2	7,4	4	21,1	4,36	0.38–50.36			
			log-Additive									
			0,1,2	27	58,7	19	41,3	2,08	0.71–6.05	0,16	60,7	0,26

*Bonferroni correction threshold is considered at 0.0083, correcting for strata and outcome (IFN viral response). Only patients with all clinical data available were included (n = 150). P-value: p-value obtained from logistic regression adjusting by HCV genotype, log10 HCV RNA viral load and IL28B-rs12979860. P-value2: p-value obtained from logistic regression without adjustment.

Dharmacon) was used as a control for putative off-targets effects in all experiments.

2.6. Drugs

3-Azido-3-deoxythymidine (1 μM, zidovudine, AZT) was purchased from Sigma-Aldrich (Madrid, Spain). Daclatasvir (25 nM, DCV) was obtained from Selleckchem. Both drugs were resuspended in phosphate buffered saline (PBS).

2.7. Quantitative RT-polymerase chain reaction (qRT-PCR)

For relative mRNA quantification, RNA was extracted using the NucleoSpin RNA II kit (Magerey-Nagel), as recommended by the manufacturer, including the DNase I treatment step. Reverse transcriptase was performed using the PrimeScript™ RT-PCR Kit (Takara). mRNA relative levels of all genes were measured by two-step quantitative RT-PCR and normalized to GAPDH mRNA expression using the DDCt method. Primers and DNA probes were purchased from Life Technologies (TaqMan gene expression assays).

Cytokine expression was evaluated by using the commercial TaqMan Human Cytokine Network array (4414255, Life Technologies), which included primers and probes for 28 different cytokine genes. mRNA relative levels of all cytokine genes were measured by two-step quantitative RT-PCR and normalized to GAPDH mRNA expression using the DDCt method.

All reactions were run and analysed on an ABI PRISM 7500 real time PCR System (Applied Biosystems).

2.8. Viral strains and infections

Envelope-deficient HIV-1 NL4-3 clone encoding IRES-luciferase was pseudotyped with VSV-G by cotransfection of HEK293T cells using polyethylenimine (PEI, Polysciences) as previously described (Pauls et al., 2014). In brief, 5×10^6 cells were plated the day before in a 75 ml flask to reach 70–80% confluence at the day of transfection. 10 μg of DNA (9 μg virus + 1 μg VSV-G) were diluted in Optimem media, mixed with 50 μl of PEI (1 μg/ml) and mixture was incubated for 20 min at RT, before adding to plated cells drop-wise. After 24 h, culture medium with serum was added and 48 h later, supernatants were harvested, filtered and stored at −80 °C. Viruses were titrated by infection of TZM cells followed by luciferase measurement in a luminometer. Huh7 and Huh7.5 cells were infected with VSV-pseudotyped NL4-3-luciferase virus and viral replication was measured two days later by quantification of luciferase production in a luminometer.

For HCV infection, we used the HCV Jc1 virus (kind gift of Dr. Charles M. Rice (The Rockefeller University) (Pietschmann et al., 2006). Transfected Huh7 cells were infected with HCV Jc1 at a MOI of 0.02 and viral replication was measured five days later by quantification of HCV RNA in infected cells. Briefly, total cellular RNA of HCV infected cells was extracted with the Quickextract™ RNA extraction kit protocol (EPICENTRE Biotechnologies) following manufacturers' protocol, including the DNAseI treatment step and extracted RNA was stored at −80 °C until analyzed. Then, 1 μg of total RNA was retro-

Table 4
Association of ADAR1 rs2229857 SNP with liver disease progression in HCV/HIV-1 coinfected patients.

Gene	SNP	Gender	Genetic model	Non-Cirrhosis (F0 to F3)	%	Cirrhosis (F4)	%	OR	95%CI	p-value	AIC	p-value2
		All (n = 142)	**Codominant**									
			C/C	61	48,4	5	31,2	1		0,32	109,1	0,27
			T/C	45	35,7	8	50	2,41	0.72–8.05			
			T/T	20	15,9	3	18,8	2,09	0.44–10			
			Dominant									
			C/C	61	48,4	5	31,2	1		0,13	107,1	0,11
			T/C-T/T	65	51,6	11	68,8	2,31	0.74–7.19			
			Recessive									
			C/C-T/C	106	84,1	13	81,2	1		0,67	109,2	0,81
			T/T	20	15,9	3	18,8	1,35	0.34–5.43			
			log-Additive									
			0,1,2	126	88,7	16	11,3	1,55	0.76–3.15	0,22	107,9	0,24
ADAR1	rs2229857	Male (n = 99)	**Codominant**									
			C/C	40	44,9	5	50	1		0,91	76,6	0,98
			T/C	34	38,2	3	30	0,78	0.17–3.68			
			T/T	15	16,9	2	20	1,17	0.19–7.13			
			Dominant									
			C/C	40	44,9	5	50	1		0,88	74,7	0,99
			T/C-T/T	49	55,1	5	50	0,9	0.24–3.45			
			Recessive									
			C/C-T/C	74	83,1	8	80	1		0,77	74,7	0,87
			T/T	15	16,9	2	20	1,28	0.23–7.17			
			log-Additive									
			0,1,2	89	89,9	10	10,1	1,02	0.41–2.51	0,96	74,7	0,93
		Female (n = 43)	**Codominant**									
			C/C	21	56,8	0	0	1		**0.005***	38,3	**0.008***
			T/C	11	29,7	5	83,3	NA	NA			
			T/T	5	13,5	1	16,7	NA	NA			
			Dominant									
			C/C	21	56,8	0	0	1		**0.001***	36,8	**0,021**
			T/C-T/T	16	43,2	6	100	NA	NA			
			Recessive									
			C/C-T/C	32	86,5	5	83,3	1		0,84	46,7	0,82
			T/T	5	13,5	1	16,7	1,29	0.1–16.41			
			log-Additive									
			0,1,2	37	86	6	14	3,93	0.96–16.06	**0,0411**	42,6	**0.008***

*Bonferroni correction threshold is considered at 0.0083, correcting for strata and outcome (cirrhosis). Only patients with fibrosis measurements were included (n = 142) P-value: p-value obtained from logistic regression adjusting by HCV genotype, log10 HCV RNA viral load and IL28B-rs12979860. P-value2: p-value obtained from logistic regression without adjustment.

transcribed to cDNA with a PrimeScript RT master mix (Takara Bio, Clontech, Inc.). cDNA was used for Taqman quantitative PCR (Premix Ex Taq (perfect real time) from Takara Bio, Clontech, Inc.) of viral HCV RNA using HCV 5′NC6 primers and probe (kindly provided by Dr. Juana Díez (Scheller et al., 2009)), normalized with the transcript GAPDH (Hs99999905_m1, Taqman Gene Expression Assay, Applied Biosystems) according to manufacturer's protocol.

2.9. Western blot

Cells were rinsed in ice-cold phosphate-buffered saline (PBS) and extracts prepared in lysis buffer (50 mM Tris HCl pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM Na3VO4, 10 mM Na β-glycerophosphate, 50 mM NaF, 5 mM Na Pyrophosphate, 270 mM sucrose and 1% Triton X-100) supplemented with protease inhibitor (Roche) and 1 mM phenylmethylsulfonyl fluoride. Lysates were subjected to SDS-PAGE and transferred to a PVDF membrane (ImmunolonP, Thermo). The following antibodies were used for immunoblotting: anti-rabbit and anti-mouse horseradish peroxidase-conjugated secondary antibodies (1:5000; Pierce); anti-human Hsp90 (1:1000; 610418, BD Biosciences, RRIB: AB_397798), anti-GAPDH (1:2500; ab9485, Abcam, RRIB: AB_307275) and anti-ADAR1 (14175, RRIB: AB_2722520), anti-MDA5 (5321, RRIB: AB_10694490), anti-phosphoSTAT1 (9167, RRIB: AB_561284),anti-RIG-I (3743, RRIB: AB_2269233), anti-phosphoIRF7 (12390, RRIB: AB_647242), anti-IRF7 (4920, RRIB: AB_2127551), anti-phosphoIRF3 (4947, RRIB: AB_2272318) and anti-IRF3 (11904, RRIB: AB_2722521) all 1:1000 from Cell Signaling.

3. Results

3.1. Polymorphisms in ADAR1 are associated to HCV infection progression in HCV/HIV-1 coinfected patients

The IFN-alpha pathway is crucial in achieving HCV viral clearance and thus, genes involved in this pathway may alter disease progression and treatment response. A cohort of HCV and HIV-1 coinfected patients (n = 155), were genotyped for ADAR1 polymorphisms rs2229857 and tested for association to treatment response. rs2229857 codes for a non-synonymous substitution, K384R, located in exon 2, between the third double-stranded RNA (dsRNA)-binding domain (dsRBD) and the first Z-DNA-binding region. Selected demographic and clinical information are summarized in Table 1 and Table 2. All patients were treated with IFN plus RBV and patients were stratified for sustained virological response (SVR) after treatment. ADAR1 rs2229857 SNP was significantly associated to IFN plus ribavirin SVR in males (adjusted p-value = 0.007, T risk allele), suggesting that ADAR1 may have a role in HCV infection outcome and response to treatment (Table 3). ADAR1 SNPs were also significantly associated to advanced fibrosis in females (Table 4, adjusted p-value = 0.001, T risk allele). Association results were replicated with an additional SNP rs1127326, which is in complete linkage disequilibrium with rs2229857 ($r^2 = 0.99$, Supplementary Tables 2 and 3), i. e., rs2229857 alleles are non-randomly associated to rs1127326 alleles.

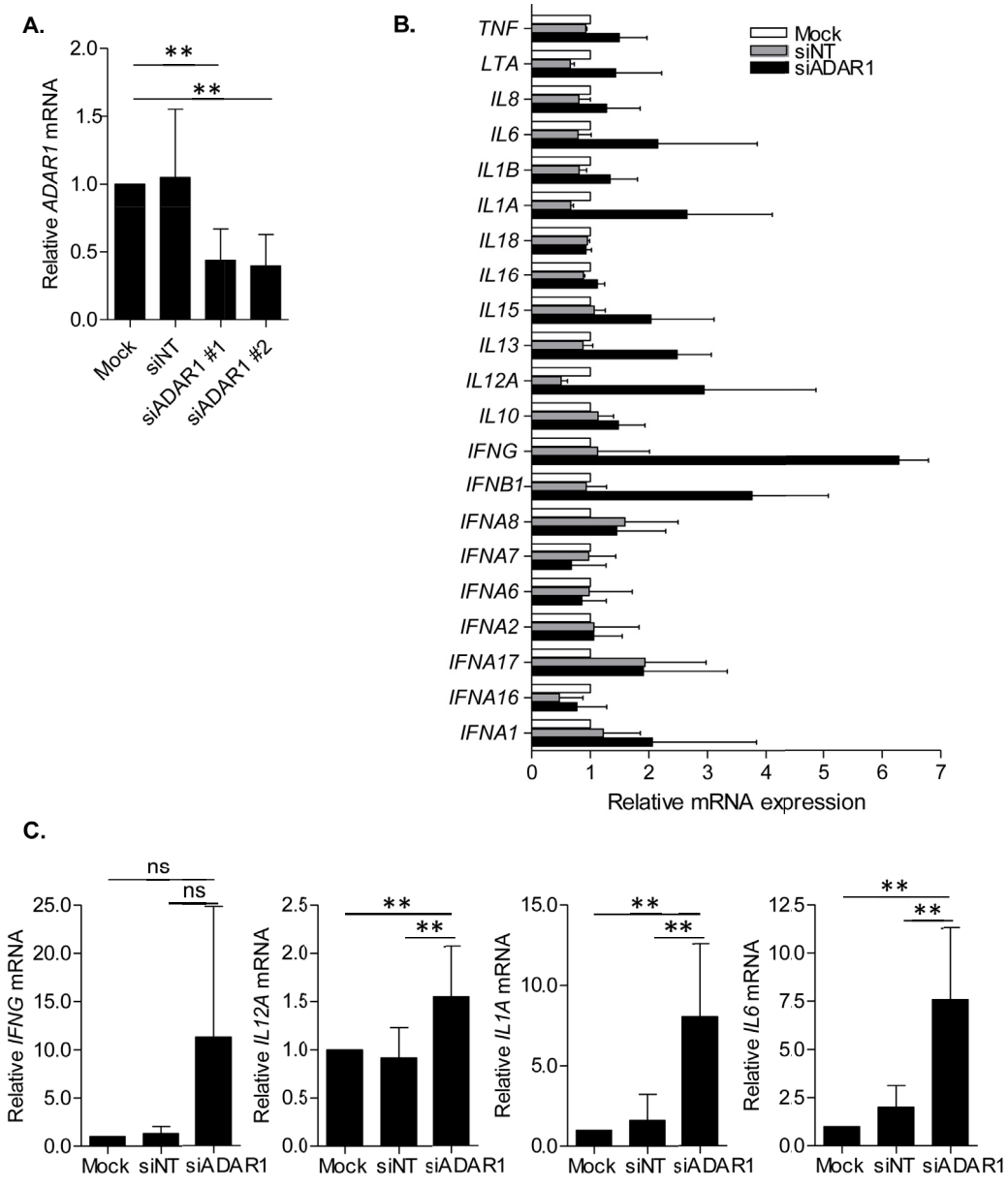

Fig. 1. ADAR1 knockdown boosts type I IFN response and blocks HIV-1 replication in primary macrophages. (A) Effective downregulation of *ADAR1* by two different siRNA in primary macrophages. Relative mRNA expression of *ADAR1* was measured by quantitative PCR and normalized to GAPDH expression. Data represents mean ± SD of 3 different donors and is normalized to Mock-transfected macrophages. **(B)** Cytokine expression profile of ADAR1 knockdown macrophages. Relative mRNA expression of the different cytokines normalized to mock-transfected macrophages is depicted. Data represents mean ± SD of 3 different donors. **(C)** Relative mRNA expression of *IFNG, IL12, IL1A* and *IL6* in ADAR1 knockdown macrophages. mRNA expression was measured by quantitative PCR and normalized to GAPDH expression. Data represents mean ± SD of 6 different donors and is normalized to Mock-transfected macrophages. ns, non-significant; ** $p < 0.005$.

3.2. ADAR1 function regulates IFN-mediated inflammation

In response to viral infection, type I IFN is produced endogenously by distinct innate immune cells, including macrophages. Thus, to further investigate the putative effect of ADAR1 as a modulator of innate immune response, ADAR1 expression was inhibited with small interfering RNA (siRNA) in monocytes and further differentiated to macrophages. Effective and specific downregulation of ADAR1 expression was achieved (Fig. 1A) and cytokine production was evaluated in ADAR1 knockdown macrophages. Significantly enhanced expression of five different IFNs and cytokine genes was observed in ADAR1 knockdown macrophages (*IFNB1*, *IFNG*, *IL12*A, *IL1A* and *IL6*) compared to mock-treated or control (siNT-treated) macrophages (Fig. 1B), an effect that was further confirmed in 6 additional donors (Fig. 1C). Upregulated cytokines are suggestive of a pro-inflammatory phenotype, indicating that inhibition of ADAR1 affects the innate immune response to viral infection. No changes where observed when using the control non-targeting siRNA.

Further characterization of the phenotype associated to ADAR1 knockdown macrophages showed increased expression of the RNA sensors MDA5 and RIG-I, increased phosphorylation of STAT1 (pSTAT1), a well-recognized marker of type I IFN induction and increased IRF7 expression (Fig. 2A). As expected, ADAR1 knockdown correlated with increased production of IFNβ (7.5-fold change, $p = 0.0388$, Fig. 2B, left panel) but also with increased expression of the IFN stimulated gene (ISG) CXCL10 (50-fold change, $p = 0.049$,

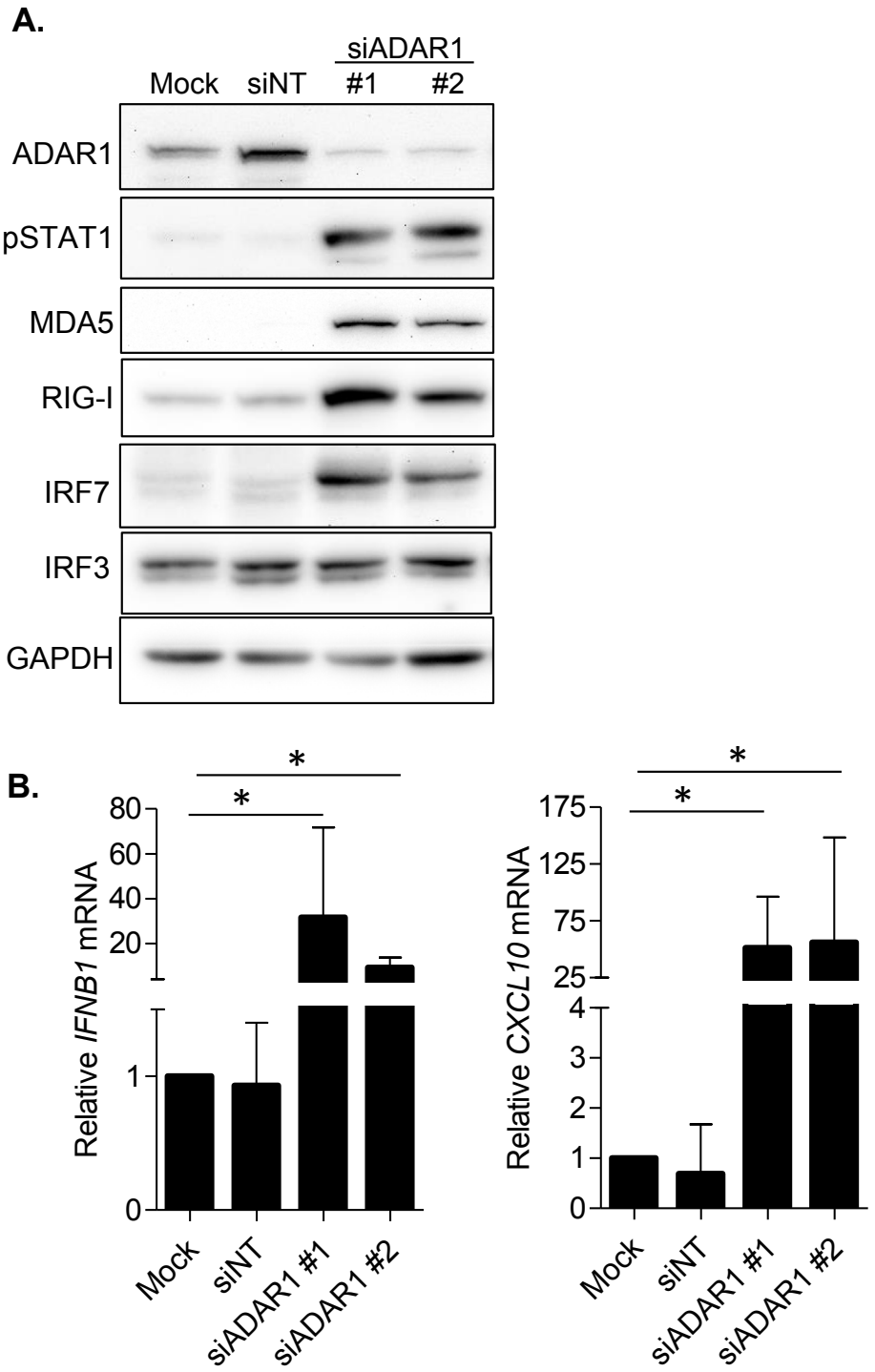

Fig. 2. ADAR1 knockdown modulates innate immune response. (A) Protein expression in ADAR1 knockdown macrophages. Western blot of ADAR1, MDA5, RIG-I, phosphorylation of STAT1 (pSTAT1), IRF3 and IRF7 in siRNA-treated macrophages. MDA5, RIG-I, pSTAT1 and IRF7 are increased in ADAR1 knockdown macrophages compared to the mock-transfected or non-targeting siRNA (siNT) treated macrophages. GAPDH was used as loading control. A representative donor is shown. **(B)** Relative mRNA expression of *IFNB1* and *CXCL10* in siRNA-treated macrophages measured by quantitative PCR and normalized to GAPDH expression. *IFNB1* and *CXCL10* gene expression was significantly enhanced in ADAR1 knockdown macrophages. Data represents mean ± SD of 3different donors and is normalized to Mock-transfected macrophages. Data represents mean ± SD of at least 3 different donors. * $p < 0.05$; *** $p < 0.0005$.

Fig. 2B, right panel), as seen in HCV/HIV-1 coinfected patients. The same effect was seen with two different siRNA targeting *ADAR1*. Interestingly, our previous data show that stimulation of macrophages with poly I:C presented a similar pattern of protein overexpression (Pujantell et al., 2016), suggesting that ADAR1 is a regulator of the RLRs-MAVS canonical pathway of innate immune activation leading to type I IFN production. Overall, these data may suggest that ADAR1 could have a role in HCV infection by regulating innate immune response.

3.3. Differential expression of ADAR1 and RNA sensors determines HIV and HCV viral susceptibility in cell lines

To further investigate the differences in the innate immune signaling pathway that might be controlled by ADAR1, expression of ADAR1 and its related RNA sensors was evaluated in different cell lines, including HuH7.5 and its parental cell line HuH7 hepatoma cell, that have been extensively used for evaluating HCV infection *in vitro*. ADAR1 total mRNA expression was relatively stable in all cell lines tested (Fig. 3A, left panel and 2B). However, MDA5 and RIG-I expression was significantly higher in HuH7 cells, compared to the others, including the hepatoma cell line HuH7.5 (Fig. 3A and B), which are

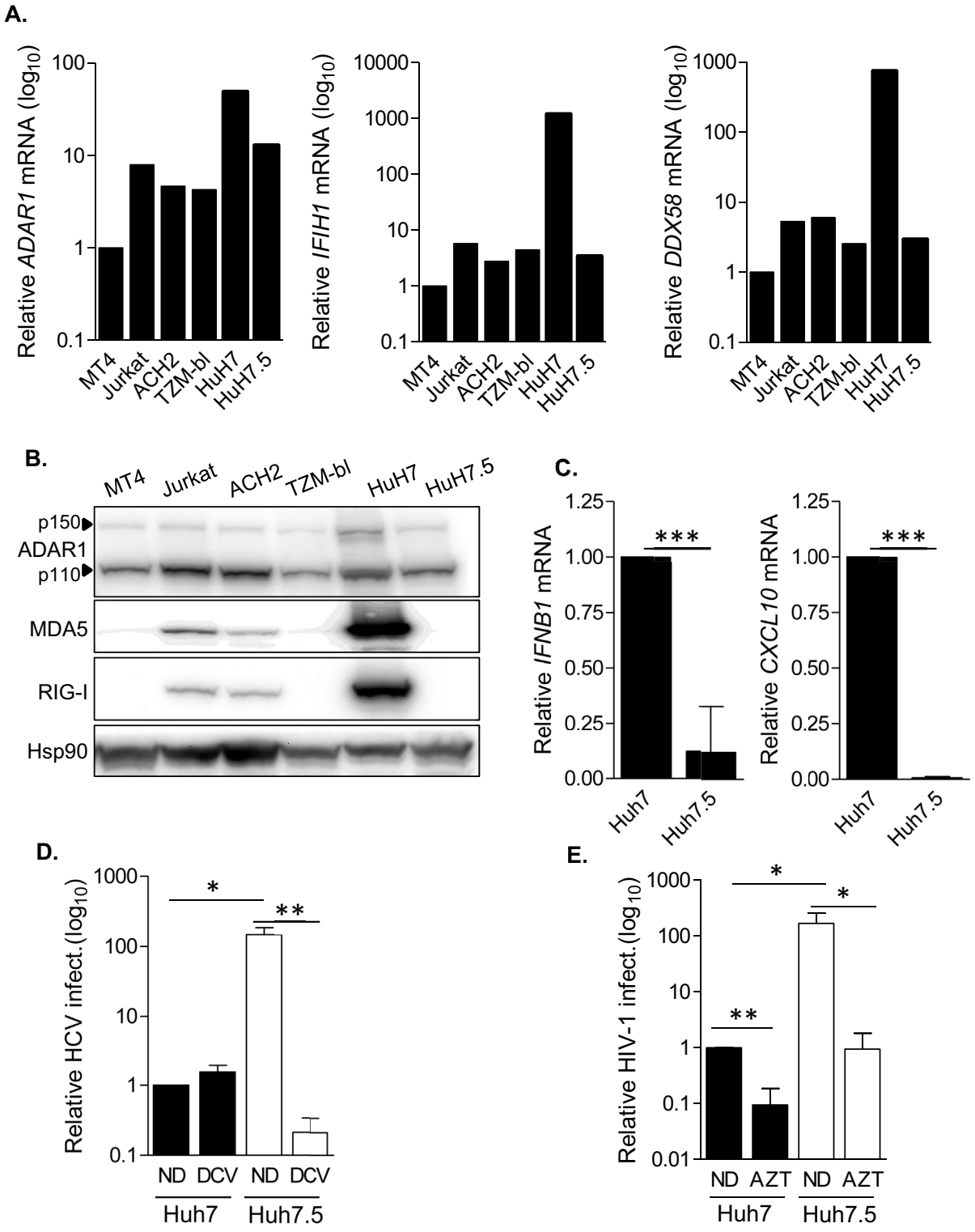

Fig. 3. Differential expression of ADAR1 and RNA sensors determines HIV and HCV viral susceptibility in cell lines. (A) Relative mRNA expression of *ADAR1*, *IFIH1* (MDA5) and *DDX58* (RIG-I) in different cell lines measured by quantitative PCR and normalized to GAPDH expression. Data is normalized to MT4 cell line. **(B)** Protein expression of ADAR1, MDA5 and RIG-I in different cell lines. Significant expression differences were observed between HuH7 and HuH7.5 cell lines. A representative blot is shown. **(C)** Relative mRNA expression of IFNB1 and CXCL10 in hepatoma cells measured by quantitative PCR and normalized to GAPDH expression. IFNB1 and CXCL10 gene expression is significantly higher in Huh7 cells. Data represents mean ± SD of 3different determinations. **(D)** Susceptibility to HIV-1 infection in HuH7 and HuH7.5 cells. Relative HIV-1 infection measured as luciferase expression is shown. AZT, zidovudine (1 μM). Data represents mean ± SD of 3 different experiments performed in triplicate and is normalized to HuH7 cells. **(E)** Susceptibility to HCV infection in HuH7 and HuH7.5 cells. Relative HCV infection measured by quantitative PCR is shown. DCV, daclatasvir (25 nM). Data represents mean ± SD of 3 different experiments performed in triplicate and is normalized to HuH7 cells. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$.

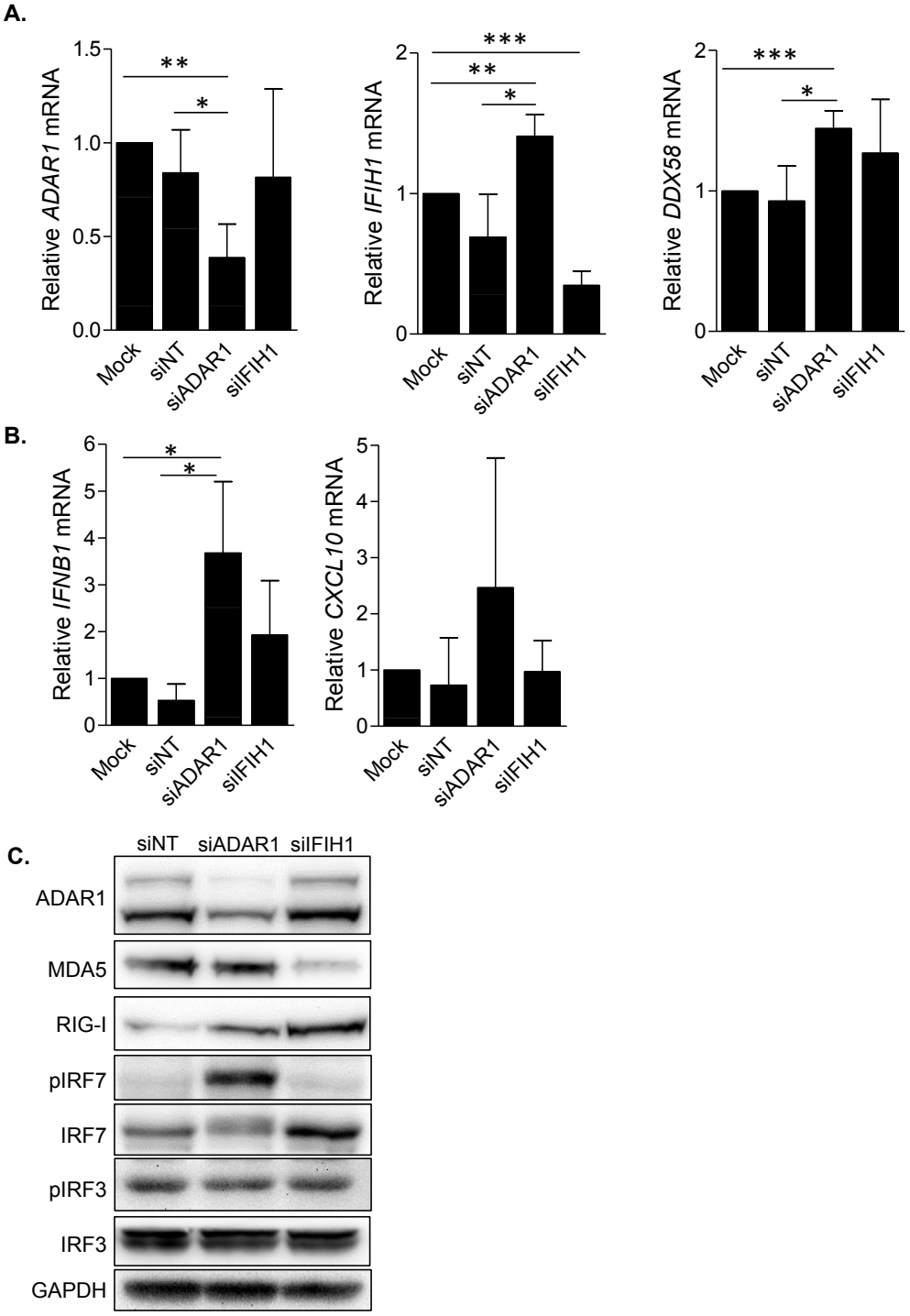

Fig. 4. ADAR1 regulates de RLRs signaling pathway in HuH7 cells. (A) Relative mRNA expression of *ADAR1*, *IFIH1* (MDA5) and *DDX58* (RIG-I) in ADAR1 and IFIH1 downregulated HuH7 cells. Gene expression is measured by quantitative PCR and normalized to GAPDH expression. ADAR1 knockdown enhanced *IFIH1* and *DDX58* gene expression. Data represents mean ± SD of at least 3 independent experiments and is normalized to Mock-transfected cells. **(B)** Relative mRNA expression of *IFNB1* and *CXCL10* in siRNA-treated HuH7 cells measured by quantitative PCR and normalized to GAPDH expression. *IFNB1* and *CXCL10* gene expression was enhanced in ADAR1 knockdown cells. Data represents mean ± SD of 3 different experiments and is normalized to Mock-transfected cells. **(C)** Protein expression of RLRs and related proteins in HuH7 cells, showing overexpression of MDA5, RIG-I, IRF7 and increased IRF7 phosphorylation consequence of ADAR1 inhibition. A representative experiment is shown. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$.

known to be RIG-I null function-wise. Interestingly, a significant difference was also observed in IFNβ and CXCL10 expression in HuH7 cells compared to Huh7.5, suggesting higher innate immune activation and type I IFN production in HuH7 cells (Fig. 3C).

Then susceptibility to HCV viral infection was evaluated in the two hepatoma cell lines and as expected,HuH7.5 cells showed 100-fold higher susceptibility to HCV infection (Fig. 3D). Previous data suggested a role for ADAR1 in HIV replication (Pujantell et al., 2016), thus, susceptibility to HIV-1 infection was also assessed obtaining similar results to HCV infection (Fig. 3E). Overall, these data suggest that innate immune effectors may be significantly modulating susceptibility to viral infection.

3.4. ADAR1 is a negative regulator of innate immune pathway in the HuH7 hepatoma cell line

In contrast to Huh7.5 cells that express undetectable levels of the RNA sensors RIG-I and MDA5, HuH7 cells have a preserved expression of innate immune effectors, a feature that may determine the outcome of HCV infection. Therefore, the role of ADAR1 and MDA5 was further investigated in HuH7 cells. Effective and specific downregulation of ADAR1 and MDA5 was achieved in HuH7 cells (Fig. 4A). ADAR1 downregulation was linked to increased *IFIH1* (MDA5) and *DDX58* (RIG-I) expression as described above for macrophages (Fig. 4A, middle and right panels), whereas no significant change was observed in MDA5

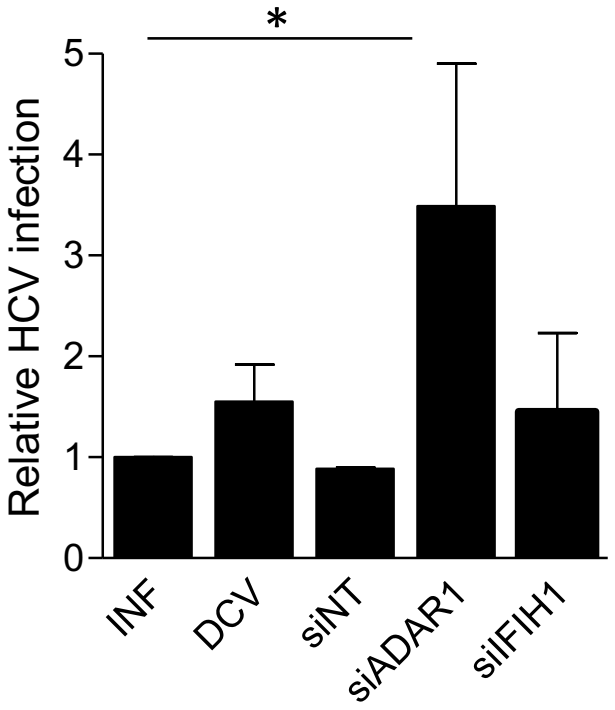

Fig. 5. ADAR1 knockdown in hepatoma cells stimulates HCV infection. Susceptibility to HCV infection in HuH7 cells treated or not with siRNA. Relative HCV infection measured by quantitative PCR is shown. Data represents mean ± SD of 3 different experiments performed in triplicate and is normalized to infected mock-transfected HuH7 cells (INFDCV, daclatasvir. * $p < 0.05$.

knockdown cells or when using a non-targeting control (Fig. 4A, left and middle panels). In addition, ADAR1 knockdown in HuH7 cells correlated also with increased production of IFNβ and increased expression of the IFN stimulated gene (ISG) CXCL10 (Fig. 4B). Importantly, the increase in IFN stimulated genes consequence of ADAR1 knockdown was also observed at protein level (Fig. 4C), showing enhanced expression of RIG-I and increased phosphorylation of IRF7, indicative of its activation as the transcription factor responsible for IFN production. We did not observe changes in IRF3 expression or phosphorylation. Increased RIG-I expression was also observed in MDA5 knockdown cells, suggesting a coordinated regulation between both RNA sensors. Conversely, no effect was seen when ADAR1 was inhibited in cells that do not express RNA sensors MDA5 and RIG-I such as Huh7.5 cells (Supplementary Fig). Overall, these data indicate that ADAR1 regulates innate immune activation and IFN production in hepatocyte-like cells and thus, can have an effect on HCV viral infection.

3.5. ADAR1 knockdown in hepatoma cells stimulates HCV infection

The role of ADAR1 and MDA5 on viral susceptibility was evaluated by testing the capacity of siRNA-treated HuH7 cells to support HCV replication. HuH7 cells were refractory to HCV infection as observed by the lack of inhibition of viral replication with DCV (Fig. 5). Knockdown of ADAR1 but not MDA5 (*IFIH1*) was able to bypass the restriction on HCV replication, supporting the notion that ADAR1 acts as an antiviral factor in HCV infection (Fig. 5).

4. Discussion

Upon HCV infection, the host induces the IFN-mediated frontline defense to limit virus replication, which is mainly mediated by type I IFN and the eliciting of its antiviral actions by inducing a wide array of ISGs. However, HCV employs diverse strategies to escape host innate immune surveillance. Indeed, although the immune response can clear HCV in some cases, virus exposure often carries forward into a chronic infection. Here, we describe the role of ADAR1 as a regulator of innate and antiviral immunity in HCV infection. ADAR1 has been proposed to be a critical suppressor of IFN responses, which protects cells from the harmful effects of excessive IFN signaling (Wang et al., 2017), and thus, ADAR1 function may affect the innate response to viral infections.

We show that in HCV/HIV-1 coinfected patients, polymorphisms within *ADAR1* gene were found significantly associated to poor clinical outcome to therapy and advanced liver fibrosis. Interestingly, rs2229857 codes for a missense mutation (K384R) that may have an impact on protein function or regulation. Our results are in accordance to previous reports where undetectable HCV RNA at was associated with an ADAR1 polymorphism (Medrano et al., 2017) (Welzel et al., 2009). The phenotype associated to ADAR1 TT allele was different between male and female individuals. Previous analysis of polymorphisms in several genes of the IFN system rs2229857 and rs1127326 revealed that host genetic factors affect HCV responsiveness to IFN therapy, in addition to other factors including HCV virus genotype, age, gender and cirrhosis status (Collazos et al., 2011; Welzel et al., 2009). HCV/HIV-1 coinfected women have more favorable HCV virological and clinical profile than men and, particularly when referring to liver disease progression. Thus, the distinct association of ADAR1 SNP in males and females may be reflecting the complex interaction of patient genetic background with viral and host factors that altogether trigger and control the outcome of HCV infection. While the analysis of polymorphisms in ADAR1 suggests that ADAR1 may play an important role in the host response to HCV infection, only limited mechanistic studies have been described that directly test this possibility, most of them based on the HCV replicon system (Appel et al., 2006). Evidence consistent with an antiviral role of ADAR1 emerged when it was found that inhibition of ADAR1 stimulated HCV viral RNA production in HCV RNA replicon-containing cells (Taylor et al., 2005). However, the availability of the JFH-1 HCV virus and Huh7 hepatoma cell system for production of infectious HCV in cell culture permits mechanistic analyses of innate antiviral responses to HCV in virus-infected cells (Lemon, 2010). Indeed, we found important differences in expression and function of innate RNA sensors between Huh7 and Huh7.5 cells, indicating that Huh7 cells may better preserve the innate immune signaling pathways. Consistent with this, silencing ADAR1 in Huh7 cells results in increased expression of innate immune sensors RIG-I and MDA5, enhanced IFN and CXCL10 production and increased IRF7 phosphorylation, presenting a similar profile to that observed in primary macrophages, and furthermore, indicating that knockdown of ADAR1 in Huh7 cells resembles innate immune activation features observed in ADAR1-associated Aircadi-Goutieres syndrome patients and in KO mouse models (Liddicoat et al., 2015; Rice et al., 2013; Wang et al., 2017).

As reported, inhibition of ADAR1 stimulated HCV viral RNA production, suggesting that ADAR1 is an important contributor to the outcome of the HCV virus–host interaction also in the infectious virus cell culture system. The exact mechanism underlying the antiviral role of ADAR1 in HCV infection is not clear. However, it is known already that ADAR1 can modulate PKR activation status in HCV-infected cells resulting in the inhibition of IFN-stimulated cellular protein synthesis but not HCV IRES-dependent viral protein synthesis (Garaigorta and Chisari, 2009). Furthermore, HCV also controls IFN production through modulation of PKR activation; PKR-mediated inhibition of IFN induction occurs in JFH1 HCV-infected Huh cells through eIF2-mediated inhibition of translation (Arnaud et al., 2010). Thus, an interesting possibility is that ADAR1 is antiviral in the context of HCV infection through a combination of mechanisms: A-to-I editing of HCV RNA reducing HCV protein expression through mutation of viral coding sequences, and editing by ADAR1 that inhibits HCV indirectly through suppression of both IFN and ISGs that will block HCV replication.

In summary, we show how ADAR1 regulates intracellular signaling

in the context of HCV infection, representing an important factor in the innate immune response. ADAR1 function is affected by the complex interaction between the patient genetic background with viral and host factors. Chronic HCV may induce aberrant innate and adaptive immune signaling and viral induced cytotoxicity and apoptosis in the hepatic environment, which together affect infection outcome and response to therapy. A better knowledge of factors triggering innate function is crucial for a better control the outcome of HCV infection and immunity (reviewed in (Horner and Gale, 2013)). This is especially relevant in the context of HCV and HIV-1 co-infected individuals were liver fibrosis progresses rapidly in partially due to heightened inflammation rather than direct HCV replication.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2018.05.012.

References

Aparicio, E., Parera, M., Franco, S., Perez-Alvarez, N., Tural, C., Clotet, B., Martinez, M.A., 2010. IL28B SNP rs8099917 is strongly associated with pegylated interferon-alpha and ribavirin therapy treatment failure in HCV/HIV-1 coinfected patients. PLoS One 5, e13771.

Appel, N., Schaller, T., Penin, F., Bartenschlager, R., 2006. From structure to function: new insights into hepatitis C virus RNA replication. J. Biol. Chem. 281, 9833–9836.

Arnaud, N., Dabo, S., Akazawa, D., Fukasawa, M., Shinkai-Ouchi, F., Hugon, J., Wakita, T., Meurs, E.F., 2011. Hepatitis C virus reveals a novel early control in acute immune response. PLoS Pathog. 7, e1002289.

Arnaud, N., Dabo, S., Maillard, P., Budkowska, A., Kalliampakou, K.I., Mavromara, P., Garcin, D., Hugon, J., Gatignol, A., Akazawa, D., Wakita, T., Meurs, E.F., 2010. Hepatitis C virus controls interferon production through PKR activation. PLoS One 5, e10575.

Badia, R., Pujantell, M., Riveira-Munoz, E., Puig, T., Torres-Torronteras, J., Marti, R., Clotet, B., Ampudia, R.M., Vives-Pi, M., Este, J.A., Ballana, E., 2016. The G1/S specific cyclin D2 is a regulator of HIV-1 restriction in non-proliferating cells. PLoS Pathog. 12, e1005829.

Ballana, E., Senserrich, J., Pauls, E., Faner, R., Mercader, J.M., Uyttebroeck, F., Palou, E., Mena, M.P., Grau, E., Clotet, B., Ruiz, L., Telenti, A., Ciuffi, A., Este, J.A., 2010. ZNRD1 (zinc ribbon domain-containing 1) is a host cellular factor that influences HIV-1 replication and disease progression. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 50, 1022–1032.

Bibert, S., Roger, T., Calandra, T., Bochud, M., Cerny, A., Semmo, N., Duong, F.H., Gerlach, T., Malinverni, R., Moradpour, D., Negro, F., Mullhaupt, B., Bochud, P.Y., Swiss Hepatitis, C.C.S., 2013. IL28B expression depends on a novel TT/-G polymorphism which improves HCV clearance prediction. J. Exp. Med. 210, 1109–1116.

Brubaker, S.W., Bonham, K.S., Zanoni, I., Kagan, J.C., 2015. Innate immune pattern recognition: a cell biological perspective. Annu. Rev. Immunol. 33, 257–290.

Chung, H., Calis, J.J.A., Wu, X., Sun, T., Yu, Y., Sarbanes, S.L., Dao Thi, V.L., Shilvock, A.R., Hoffmann, H.H., Rosenberg, B.R., Rice, C.M., 2018. Human ADAR1 prevents endogenous RNA from triggering translational shutdown. Cell 172, 811–824 e814.

Collazos, J., Carton, J.A., Asensi, V., 2011. Gender differences in liver fibrosis and hepatitis C virus-related parameters in patients coinfected with human immunodeficiency virus. Curr. HIV Res. 9, 339–345.

Franco, S., Aparicio, E., Parera, M., Clotet, B., Tural, C., Martinez, M.A., 2014. IFNL4 ss469415590 variant is a better predictor than rs12979860 of pegylated interferon-alpha/ribavirin therapy failure in hepatitis C virus/HIV-1 coinfected patients. Aids 28, 133–136.

Gao, X., Starmer, J., Martin, E.R., 2008. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genet. Epidemiol. 32, 361–369.

Garaigorta, U., Chisari, F.V., 2009. Hepatitis C virus blocks interferon effector function by inducing protein kinase R phosphorylation. Cell Host Microbe 6, 513–522.

Ge, D., Fellay, J., Thompson, A.J., Simon, J.S., Shianna, K.V., Urban, T.J., Heinzen, E.L., Qiu, P., Bertelsen, A.H., Muir, A.J., Sulkowski, M., McHutchison, J.G., Goldstein, D.B., 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461, 399–401.

George, C.X., John, L., Samuel, C.E., 2014. An RNA editor, adenosine deaminase acting on double-stranded RNA (ADAR1). J. Interferon Cytokine Res. Off. J. Int. Soc. Interferon Cytokine Res. 34, 437–446.

George, C.X., Ramaswami, G., Li, J.B., Samuel, C.E., 2016. Editing of cellular self-RNAs by adenosine deaminase ADAR1 suppresses innate immune stress responses. J. Biol. Chem. 291, 6158–6168.

Gonzalez, J.R., Armengol, L., Sole, X., Guino, E., Mercader, J.M., Estivill, X., Moreno, V., 2007. SNPassoc: an R package to perform whole genome association studies. Bioinformatics 23, 644–645.

Heim, M.H., 2013. Innate immunity and HCV. J. Hepatol. 58, 564–574.

Horner, S.M., Gale Jr., M., 2009. Intracellular innate immune cascades and interferon defenses that control hepatitis C virus. J. Interferon Cytokine Res. Off. J. Int. Soc. Interferon Cytokine Res. 29, 489–498.

Horner, S.M., Gale Jr., M., 2013. Regulation of hepatic innate immunity by hepatitis C virus. Nat. Med. 19, 879–888.

Jones, C.T., Catanese, M.T., Law, L.M., Khetani, S.R., Syder, A.J., Ploss, A., Oh, T.S., Schoggins, J.W., MacDonald, M.R., Bhatia, S.N., Rice, C.M., 2010. Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system. Nat. Biotechnol. 28, 167–171.

Lauer, G.M., Walker, B.D., 2001. Hepatitis C virus infection. N. Engl. J. Med. 345, 41–52.

Lavanchy, D., 2009. The global burden of hepatitis C. Liver Int. Off. J. Int. Assoc. Study Liv. 29 (Suppl. 1), 74–81.

Lemon, S.M., 2010. Induction and evasion of innate antiviral responses by hepatitis C virus. J. Biol. Chem. 285, 22741–22747.

Liddicoat, B.J., Piskol, R., Chalk, A.M., Ramaswami, G., Higuchi, M., Hartner, J.C., Li, J.B., Seeburg, P.H., Walkley, C.R., 2015. RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself. Science 349, 1115–1120.

Loo, Y.M., Owen, D.M., Li, K., Erickson, A.K., Johnson, C.L., Fish, P.M., Carney, D.S., Wang, T., Ishida, H., Yoneyama, M., Fujita, T., Saito, T., Lee, W.M., Hagedorn, C.H., Lau, D.T., Weinman, S.A., Lemon, S.M., Gale Jr., M., 2006. Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. Proc. Natl. Acad. Sci. U. S. A. 103, 6001–6006.

Mannion, N.M., Greenwood, S.M., Young, R., Cox, S., Brindle, J., Read, D., Nellaker, C., Vesely, C., Ponting, C.P., McLaughlin, P.J., Jantsch, M.F., Dorin, J., Adams, I.R., Scadden, A.D., Ohman, M., Keegan, L.P., O'Connell, M.A., 2014. The RNA-editing enzyme ADAR1 controls innate immune responses to RNA. Cell Rep. 9, 1482–1494.

Medrano, L.M., Berenguer, J., Jimenez-Sousa, M.A., Aldamiz-Echevarria, T., Tejerina, F., Diez, C., Vigon, L., Fernandez-Rodriguez, A., Resino, S., 2017. ADAR1 polymorphisms are related to severity of liver fibrosis in HIV/HCV-coinfected patients. Sci. Rep. 7, 12918.

Nishikura, K., 2016. A-to-I editing of coding and non-coding RNAs by ADARs. Nat. Rev. Mol. Cell Biol. 17, 83–96.

Pauls, E., Ruiz, A., Badia, R., Permanyer, M., Gubern, A., Riveira-Munoz, E., Torres-Torronteras, J., Alvarez, M., Mothe, B., Brander, C., Crespo, M., Menendez-Arias, L., Clotet, B., Keppler, O.T., Marti, R., Posas, F., Ballana, E., Este, J.A., 2014. Cell cycle control and HIV-1 susceptibility are linked by CDK6-dependent CDK2 phosphorylation of SAMHD1 in myeloid and lymphoid cells. J. Immunol. 193, 1988–1997.

Pietschmann, T., Kaul, A., Koutsoudakis, G., Shavinskaya, A., Kallis, S., Steinmann, E., Abid, K., Negro, F., Dreux, M., Cosset, F.L., Bartenschlager, R., 2006. Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. Proc. Natl. Acad. Sci. U. S. A. 103, 7408–7413.

Prokunina-Olsson, L., Muchmore, B., Tang, W., Pfeiffer, R.M., Park, H., Dickensheets, H., Hergott, D., Porter-Gill, P., Mumy, A., Kohaar, I., Chen, S., Brand, N., Tarway, M., Liu, L., Sheikh, F., Astemborski, J., Bonkovsky, H.L., Edlin, B.R., Howell, C.D., Morgan, T.R., Thomas, D.L., Rehermann, B., Donnelly, R.P., O'Brien, T.R., 2013. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat. Genet. 45, 164–171.

Pujantell, M., Badia, R., Ramirez, C., Puig, T., Clotet, B., Ballana, E., Este, J.A., Riveira-Munoz, E., 2016. Long-term HIV-1 infection induces an antiviral state in primary macrophages. Antivir. Res. 133, 145–155.

Rauch, A., Kutalik, Z., Descombes, P., Cai, T., Di Iulio, J., Mueller, T., Bochud, M., Battegay, M., Bernasconi, E., Borovicka, J., Colombo, S., Cerny, A., Dufour, J.F., Furrer, H., Gunthard, H.F., Heim, M., Hirschel, B., Malinverni, R., Moradpour, D., Mullhaupt, B., Witteck, A., Beckmann, J.S., Berg, T., Bergmann, S., Negro, F., Telenti, A., Bochud, P.Y., Swiss Hepatitis, C.C.S., Swiss, H.I.V.C.S., 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 138, 1338–1345 1345 e1331-1337.

Rice, G.I., Forte, G.M., Szynkiewicz, M., Chase, D.S., Aeby, A., Abdel-Hamid, M.S., Ackroyd, S., Allcock, R., Bailey, K.M., Balottin, U., Barnerias, C., Bernard, G., Bodemer, C., Botella, M.P., Cereda, C., Chandler, K.E., Dabydeen, L., Dale, R.C., De Laet, C., De Goede, C.G., Del Toro, M., Effat, L., Enamorado, N.N., Fazzi, E., Gener, B., Haldre, M., Lin, J.P., Livingston, J.H., Lourenco, C.M., Marques Jr., W., Oades, P., Peterson, P., Rasmussen, M., Roubertie, A., Schmidt, J.L., Shalev, S.A., Simon, R., Spiegel, R., Swoboda, K.J., Temtamy, S.A., Vassallo, G., Vilain, C.N., Vogt, J., Wermenbol, V., Whitehouse, W.P., Soler, D., Olivieri, I., Orcesi, S., Aglan, M.S., Zaki, M.S., Abdel-Salam, G.M., Vanderver, A., Kisand, K., Rozenberg, F., Lebon, P., Crow, Y.J., 2013. Assessment of interferon-related biomarkers in Aicardi-Goutieres syndrome associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: a case-control study. Lancet Neurol. 12, 1159–1169.

Samuel, C.E., 2011. Adenosine deaminases acting on RNA (ADARs) are both antiviral and proviral. Virology 411, 180–193.

Sarasin-Filipowicz, M., Oakeley, E.J., Duong, F.H.T., Christen, V., Terracciano, L., Filipowicz, W., Heim, M.H., 2008. Interferon signaling and treatment outcome in

chronic hepatitis C. Proc. Natl. Acad. Sci. U. S. A. 105, 7034–7039.
Scheller, N., Mina, L.B., Galao, R.P., Chari, A., Gimenez-Barcons, M., Noueiry, A., Fischer, U., Meyerhans, A., Diez, J., 2009. Translation and replication of hepatitis C virus genomic RNA depends on ancient cellular proteins that control mRNA fates. Proc. Natl. Acad. Sci. U. S. A. 106, 13517–13522.
Seeff, L.B., 2009. The history of the "natural history" of hepatitis C (1968-2009). Liver Int. Off. J. Int. Assoc. Study Liv. 29 (Suppl. 1), 89–99.
Seki, E., Brenner, D.A., 2008. Toll-like receptors and adaptor molecules in liver disease: update. Hepatology 48, 322–335.
Song, C., Sakurai, M., Shiromoto, Y., Nishikura, K., 2016. Functions of the RNA editing enzyme ADAR1 and their relevance to human diseases. Genes 7.
Suppiah, V., Moldovan, M., Ahlenstiel, G., Berg, T., Weltman, M., Abate, M.L., Bassendine, M., Spengler, U., Dore, G.J., Powell, E., Riordan, S., Sheridan, D., Smedile, A., Fragomeli, V., Muller, T., Bahlo, M., Stewart, G.J., Booth, D.R., George, J., 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat. Genet. 41, 1100–1104.
Tamura, T., Yanai, H., Savitsky, D., Taniguchi, T., 2008. The IRF family transcription factors in immunity and oncogenesis. Annu. Rev. Immunol. 26, 535–584.
Tanaka, Y., Nishida, N., Sugiyama, M., Kurosaki, M., Matsuura, K., Sakamoto, N., Nakagawa, M., Korenaga, M., Hino, K., Hige, S., Ito, Y., Mita, E., Tanaka, E., Mochida, S., Murawaki, Y., Honda, M., Sakai, A., Hiasa, Y., Nishiguchi, S., Koike, A., Sakaida, I., Imamura, M., Ito, K., Yano, K., Masaki, N., Sugauchi, F., Izumi, N., Tokunaga, K., Mizokami, M., 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat. Genet. 41, 1105–1109.
Taylor, D.R., Puig, M., Darnell, M.E., Mihalik, K., Feinstone, S.M., 2005. New antiviral pathway that mediates hepatitis C virus replicon interferon sensitivity through ADAR1. J. Virol. 79, 6291–6298.
Thomas, D.L., Thio, C.L., Martin, M.P., Qi, Y., Ge, D., O'Huigin, C., Kidd, J., Kidd, K., Khakoo, S.I., Alexander, G., Goedert, J.J., Kirk, G.D., Donfield, S.M., Rosen, H.R., Tobler, L.H., Busch, M.P., McHutchison, J.G., Goldstein, D.B., Carrington, M., 2009. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461, 798–801.
Wang, G., Wang, H., Singh, S., Zhou, P., Yang, S., Wang, Y., Zhu, Z., Zhang, J., Chen, A., Billiar, T., Monga, S.P., Wang, Q., 2015. ADAR1 prevents liver injury from inflammation and suppresses interferon production in hepatocytes. Am. J. Pathol. 185, 3224–3237.
Wang, N., Liang, Y., Devaraj, S., Wang, J., Lemon, S.M., Li, K., 2009. Toll-like receptor 3 mediates establishment of an antiviral state against hepatitis C virus in hepatoma cells. J. Virol. 83, 9824–9834.
Wang, Q., Li, X., Qi, R., Billiar, T., 2017. RNA editing, ADAR1, and the innate immune response. Genes 8.
Welzel, T.M., Morgan, T.R., Bonkovsky, H.L., Naishadham, D., Pfeiffer, R.M., Wright, E.C., Hutchinson, A.A., Crenshaw, A.T., Bashirova, A., Carrington, M., Dotrang, M., Sterling, R.K., Lindsay, K.L., Fontana, R.J., Lee, W.M., Di Bisceglie, A.M., Ghany, M.G., Gretch, D.R., Chanock, S.J., Chung, R.T., O'Brien, T.R., Group, H.-C.T., 2009. Variants in interferon-alpha pathway genes and response to pegylated interferon-Alpha2a plus ribavirin for treatment of chronic hepatitis C virus infection in the hepatitis C antiviral long-term treatment against cirrhosis trial. Hepatology 49, 1847–1858.