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YAP mediates HIV-related liver fibrosis

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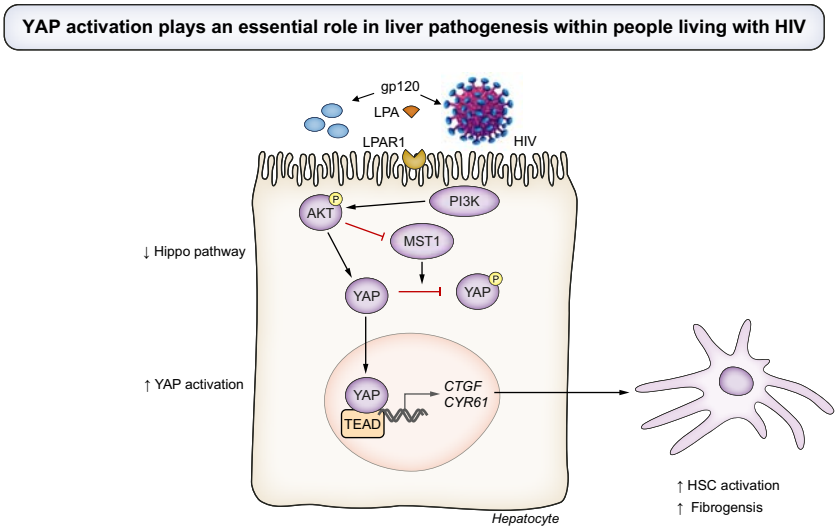
Graphical abstract



Highlights:

- HIV infection induced YAP activation in hu-mice, in human precision-cut liver slices, and *in vitro* 2 or 3D liver models.
- HIV infection upregulated circulating YAP-related proteins in people living with HIV.
- HIV protein gp120 induced the upregulation of YAP-related genes and the expression of profibrotic markers.
- LPA, PI3K, and AKT regulate HIV-induced YAP activation in Huh7 cells.

Impact and implications:

There are currently no FDA-approved treatments for cirrhosis, while liver disease is the second leading cause of mortality among people living with HIV after AIDS. Increased lysophosphatidic acid concentrations and AKT activation after HIV infection found in recent work suggest that the Hippo pathway may be a key regulator of HIV-related fibrogenesis. By linking lysophosphatidic acid signaling, YAP activation, and HIV-related fibrogenesis, this mechanism presents a target for future research into therapeutic interventions for not only HIV but also other liver diseases, *e.g.* metabolic dysfunction- or alcohol-associated liver disease.

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Research article

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YAP mediates HIV-related liver fibrosis

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Background & Aims: HIV accelerates liver fibrosis attributable to multiple etiologies, including HCV, HBV, and steatotic liver disease. Evidence also suggests that HIV infection itself is associated with liver fibrogenesis. Recent studies have implicated Yes-associated protein 1 (YAP1) and the upstream lysophosphatidic acid (LPA)/PI3K/AKT pathway as critical regulators of hepatic fibrogenesis, and suggest a connection to HIV-related liver fibrosis. However, the relationship between YAP/PI3K/AKT pathway activation and HIV-related liver fibrosis remains uncertain.

Methods: qPCR, western blot, immunofluorescence, and ELISA (replicates n ≥3) were performed in an unbiased humanized mouse model (NRG-hu HSC mice, n = 6), the precision cut liver slice *ex vivo* model, and both traditional *in vitro* models as well as a 3D spheroid system.

Results: YAP target gene mRNA and protein levels (ANKRD, CTGF, CYR61) were upregulated across all models exposed to HIV. Humanized mice infected with HIV had significant increases in the percentage of YAP-positive nuclei (2.2-fold) and the percentage area of Sirius Red collagen staining (3.3-fold) compared to control mice. Serum concentrations of LPA were increased 5.8-fold in people living with HIV compared to healthy controls. Modulation of LPAR1, PI3K, and AKT by either inhibitors or small-interfering RNAs abrogated the fibrotic effects of HIV exposure and downregulated YAP target genes within cultured liver cells.

Conclusions: The LPAR/PI3K/AKT axis is vital for the activation of YAP and hepatic fibrogenesis due to HIV infection. This novel mechanistic insight suggests new pharmacologic targets for treatment of liver fibrosis in people living with HIV.

Introduction

Currently over one million people are infected with HIV-1 in the United States alone.[1] While once considered a fatal diagnosis, HIV is now widely recognized as a chronic illness due to the successful implementation of antiretroviral therapy (ART). However, among people living with HIV (PLWH), liver disease continues to be a leading cause of non-AIDS-related mortality.[2–4] It has been well established that HIV infection accelerates the progression of liver fibrosis, and many studies have suggested that HIV infection itself is a cause of hepatic fibrosis.[5–8,11] Despite this focus, the precise mechanisms that connect HIV and hepatic fibrogenesis remain incompletely understood.

The Hippo pathway is responsible for regulating organ size, regeneration, and cell polarity and has become a focus of scientific inquiry due to its known role in liver fibrogenesis.[9–11] Key players in the Hippo pathway, mammalian Ste20-like kinases 1/2 (MST1/2) and large tumor suppressor 1/2 (LATS1/2) form a complex to prevent signal propagation through downstream effectors, such as Yes-associated protein 1 (YAP), which is critical to the development of fibrosis in many organs, including the liver.[12–14] Many components of the pathway are secreted from the cell including proteins like the well-known biomarkers for fibrosis progression, connective tissue growth factor (CTGF) and MST1.[15,16] While the Hippo pathway is active YAP is phosphorylated, preventing translocation to the nucleus and marking YAP for ubiqitination.[17] Current knowledge of the Hippo pathway implicates three upstream mediators driving normal YAP regulation. These mediators include growth factor signaling through tyrosine kinases such as PI3K and AKT and G protein-coupled receptor ligands such as lysophosphatidic acid (LPA).[18–20]

The PI3K and AKT pathway is an upstream regulator of the Hippo pathway, participating in cell growth and survival responses. Activated AKT will prevent the phosphorylation of MST1/2 and LATS1/2 and subsequent YAP degradation.[21] Due to the widespread prevalence of constitutively active PI3K/AKT/YAP mutants among various cancers, their relationship is well documented.[22,23] However, in the context of HIV and fibrosis, HIV infection itself directly increases AKT activity in primary human macrophages.[24] LPA and lysophosphatidic acid receptors (LPARs) are also particularly relevant. Elevated LPA levels have been correlated with fibrosis stage in both humans and rats infected with HCV.[25,26] Moreover, LPA signaling is involved in the fibropathogenesis of both hepatocellular carcinoma (HCC) and cholangiocarcinoma.[27] In addition to its

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involvement in hepatic fibrogenesis, recent work has demonstrated a connection between LPA signaling and HIV infection. A recent study found that serum LPA concentrations are elevated in HIV-infected patients[28] and that HIV-1 directly engages CXCR4 and CCR5, both of which are GPCRs similar to the LPAR family.[29–33] Together, these studies suggest that LPA may be key in a molecular mechanism that explains the exacerbated fibrosis observed clinically in persons living with HIV. While the mechanistic relationship between HIV and the Hippo pathway remains unknown, these studies cumulatively suggest that HIV modulates the activity of YAP and a subsequent profibrogenic program within the liver.

In the present study, we examined the role of YAP/LPA and LPAR1 in HIV-related liver fibrosis and found that LPAR1 mediates YAP activation and induces hepatic stellate cell (HSC) activation. In addition, we found that HIV either directly interacts with or indirectly upregulates several pathways upstream of YAP, leading to a Hippo "off" state. In this state, inactive YAP from the cytoplasm translocates to and accumulates within the nucleus, enabling the upregulation of multiple target genes that constitute a profibrogenic program. Ultimately, the Hippo pathway represents an ideal candidate for further investigation to integrate the mechanisms behind the accelerated development of fibrosis in PLWH and to identify possible targets for pharmacologic intervention.

Materials and methods

Please see the supplementary materials file for a detailed description of the models and techniques used in this article.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2019 and GraphPad Prism 8 (GraphPad software, San Diego, CA). Data are presented as mean ± SEM from at least three independent experiments unless otherwise stated in the Fig. legends. p values were generally calculated using the Mann-Whitney U test and ANOVA. Non-parametric tests were preferred, though Student's t test and ANOVA (for normally distributed data) were used for data sets with sample sizes less than six. p <0.05 was considered statistically significant.

Results

HIV-1 infection induced YAP activation, an upregulation in canonically profibrotic genes, and signs of hepatitis and liver fibrosis in humanized mice

To investigate the contribution of YAP to HIV-related fibrogenesis, we analyzed variations in mRNA and protein expression in a humanized mouse model (hu-mice) reconstituted with a human immune system. NRG mice were reconstituted with CD34+ progenitor cells isolated from human fetal livers and pluripotent hematopoietic stem cells to generate NRG-hu HSC mice, which were infected with HIV-1 for 12-13 weeks, as described in detail in our previous work.[34–36] Quantitative reverse-transcription PCR (qRT-PCR) results from HIV-infected mice demonstrated increases in the mRNA expression of *Yap* and YAP-dependent genes (*Cyr61*, *Ctgf*) and the pro-fibrotic marker *Col1A1* compared to control mice injected with 293T supernatant (Fig. 1A). Murine primers for transcripts of interest were used in qRT-PCR of humanized mouse samples; human primers did not generate any detectable signal. Western blot data confirmed the 3-fold increase in total Yap and Cyr61 protein levels (Fig. 1B). While total pYap (Ser127) levels also increased, the ratio of pYap to Yap decreased in HIV-infected mice. In addition, we detected a considerable induction of Akt and pAkt at the protein level in liver cell lysates of HIV-infected mice compared to controls, suggesting upregulated Akt activation within liver cell populations during HIV infection.

Analysis of liver sections from mice infected with HIV for 12 weeks revealed a substantial increase in Yap immunostaining compared to uninfected control mice (Fig. 1C). These sections also showed that Yap induction was associated with Yap nuclear translocation (Fig. 1C). While there is no hepatocyte-specific staining in these images, hepatocytes will comprise roughly 80% of cells in murine liver samples.[37] This potentially suggests that our conclusions from the presented data also apply to all liver cells. Besides inducing Yap activation, hu-mice infected with HIV also had elevated Ctgf and significantly increased Lpa and Cyr61 serum levels compared with uninfected control mice (Fig. 1D).

These findings coincide with increased total liver collagen content, specifically in portal areas (demonstrated by Sirius Red staining; Fig. 1E), and confirm our previously reported findings of significant increases in serum alanine aminotransferase and inflammation in liver sections of HIV-infected mice compared with control mice.[34] Collectively, our *in vivo* mouse data indicate that Yap activity is induced in the HIV humanized mouse model and is associated with the activation of Akt and increased collagen expression and deposition surrounding the portal tract.

HIV infection upregulated circulating YAP-related proteins in PLWH, in pHHs, and in PCLS

To validate our findings in humans, we performed analogous experiments using the serum of PLWH and primary human hepatocytes (pHHs) from three independent donors exposed to HIV. We observed a significant increase in CYR61, CTGF, and LPA serum levels within HIV-infected patients prior to ART. MST1 serum levels were significantly reduced in those same patients in comparison with healthy controls (Fig. 2A).

Exposure to HIV X4/R5 dual tropic (R3)[38] for 72 h produced no observable changes in pHH *YAP* mRNA levels compared to mock infection with 293T supernatant. However, significant increases in the mRNA expression of the YAP-dependent genes *CTGF, CYR61*, and *ANKRD* were detected relative to mock-treated cells (Fig. 2B). At the protein level, HIV exposure prompted a 3- to 6-fold induction of AKT and pAKT, and the YAP-responsive protein CYR61. However, there was no change in the expression of YAP itself. A reduction in pYAP, including the ratio of pYAP/YAP, was also interpreted as confirmation of YAP activation (Fig. 2C).

With this data, we confirmed that HIV infection is associated with the activation of the AKT/YAP pathway in pHHs. However, patient-derived cell lines, isolated and cultured *ex vivo*, cannot fully replicate the complex liver microenvironment; for example, they do not account for cell-cell communication via soluble mediators and direct contact. Even hu-mouse models may lack some of the mechanisms involved in HIV infection. Precision cut liver slices (PCLS) are an improved *ex vivo* model derived from healthy patients. This model enables us to analyze various

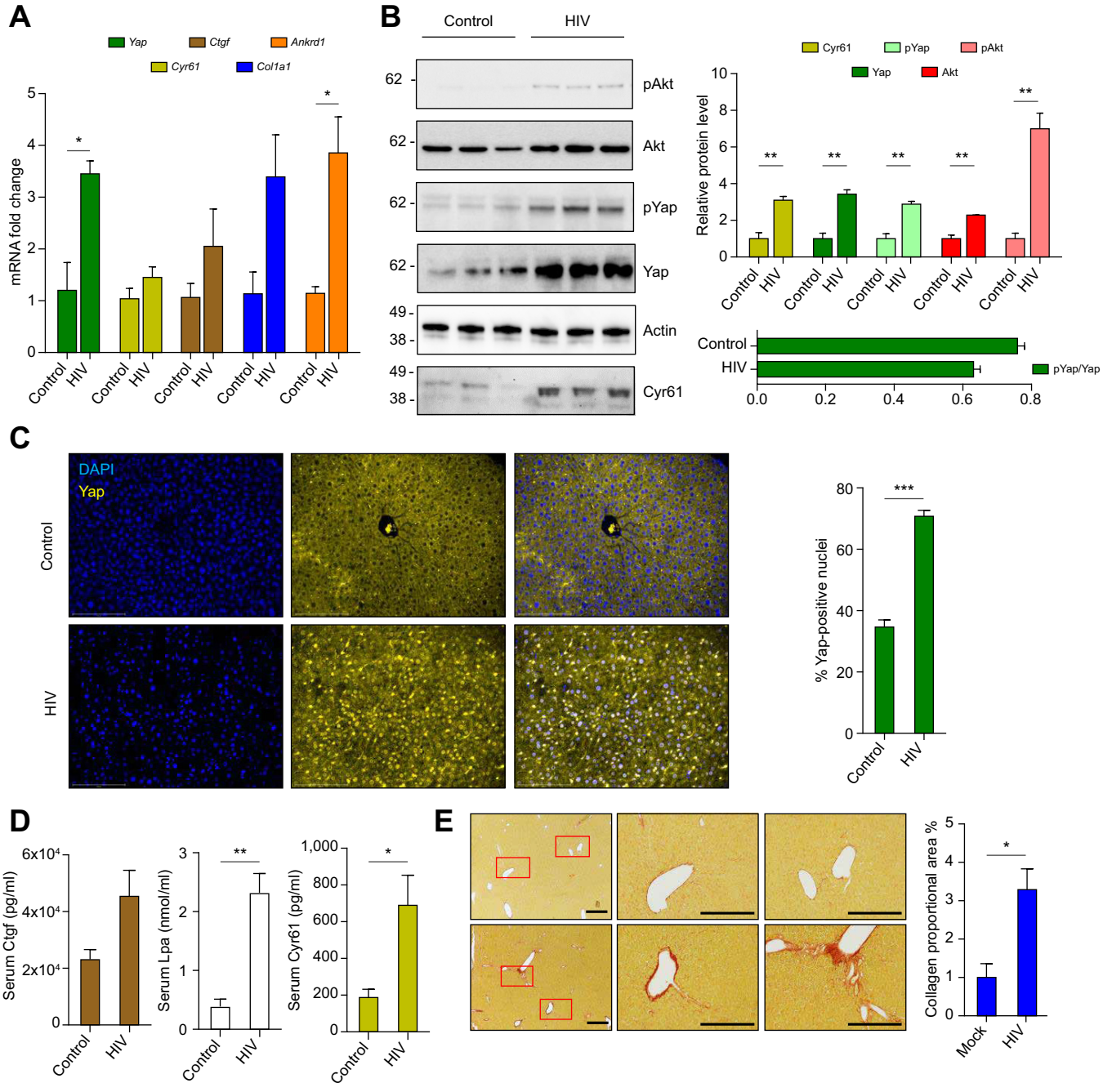

Fig. 1. HIV exposure upregulated YAP-related genes in the livers of a humanized murine model. (A) *Yap*-related gene expression was measured via qRT-PCR in liver specimens derived from Hu-mice 12 weeks post HIV infection. Levels of significance: $p_{(Yap)} = 0.019$, $p_{(Cyr61)} = 0.224$, $p_{(Ctgf)} = 0.271$, $p_{(Col1a1)} = 0.069$, $p_{(Ankrd)} = 0.018$ (Student's *t* test). (B) Proteomic confirmation of these findings within the same samples was completed via western blots and presented as relative signal strength generated via computer analysis (Image Studio). The ratio of relative signal strength between pYap and Yap is also shown. Levels of significance: $p_{(Cyr61)} = 0.006$, $p_{(Yap)} = 0.003$, $p_{(pYap)} = 0.004$, $p_{(Akt)} = 0.003$, $p_{(pAkt)} = 0.003$, $p_{(pYap/Yap)} = 0.081$ (Student's *t* test). (C) Immunofluorescent staining of Yap (CY5, yellow) nuclear translocation within the same murine liver specimens examined with qRT-PCR and western blot. Images are presented at 4X magnification. Percentage of Yap-positive nuclei are graphed as the mean colocalization ratios with DNA (DAPI, blue) in six images per mouse (n = 3 per condition). Levels of significance: p <0.001 (Mann-Whitney *U* test). (D) Mean CTGF, LPA, and CYR61 serum levels determined by ELISA in control (n = 4) and HIV-infected mice (n = 7). Levels of significance: $p_{(Ctgf)} = 0.083$, $p_{(Lpa)} = 0.006$, $p_{(Cyr61)} = 0.012$ (Mann-Whitney *U* test). E) Sirius Red staining of collagen in Hu-mice livers 12 weeks post HIV infection and control Hu-mice samples. Sirus Red staining is quantified as the mean area percentage of collagen deposition for each condition. Images are presented at 4X and 20X magnification. Scale bar = 300 μm. Levels of significance: $p = 0.031$ (Mann-Whitney *U* test). Data are presented as mean ± SEM (n = 3 biological replicates). qRT-PCR, quantitative reverse-transcription PCR.

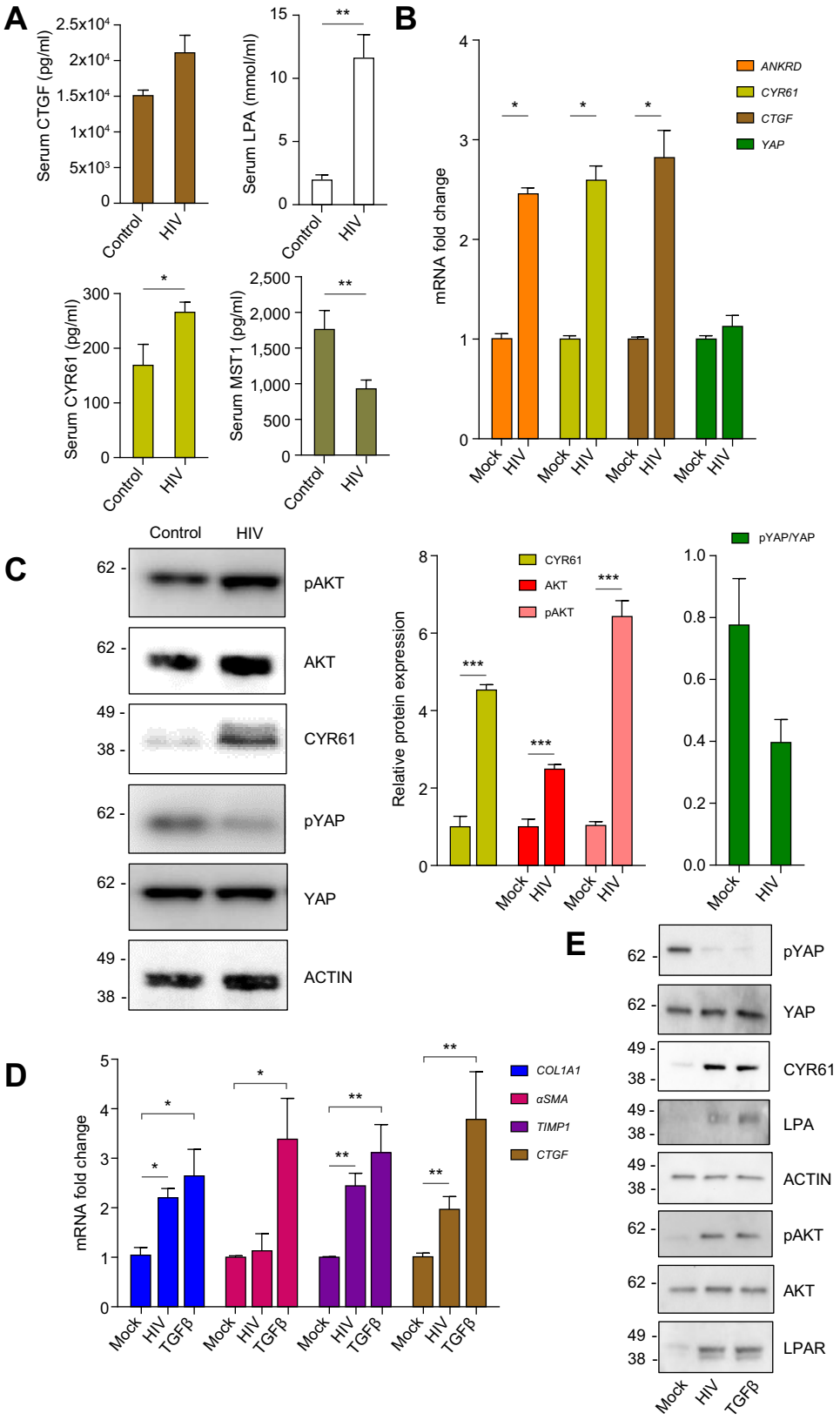

Fig. 2. HIV infection upregulated YAP-related proteins within serum from people living with HIV, pHHs, and PCLS exposed to HIV. (A) Mean CTGF, LPA, CYR61, and MST1 serum levels in controls (n = 10) and in people living with HIV pre-antiretroviral treatment (n = 29). Levels of significance: $p_{(CTGF)} = 0.536$, $p_{(LPA)} = 0.007$, $p_{(CYR61)} = 0.038$, $p_{(MST1)} = 0.002$ (Mann-Whitney *U* test). (B) qRT-PCR analysis of YAP-related gene expression in pHHs from three different donors exposed to 293T supernatant (mock) or HIV for 72 h. Values were normalized to *GAPDH*. Levels of significance: $p_{(ANKRD)} = 0.013$, $p_{(CYR61)} = 0.013$, $p_{(CTGF)} = 0.013$, $p_{(YAP)} = 0.216$

cell types, including hepatocytes and HSCs, within preserved hepatic architecture. To examine the viability of HIV(R3) infection in this model, PCLS were incubated with a HIV1 R3[38] strain for 4 days. We confirmed that HIV replication in PCLS increased in a time-dependent manner by measuring HIV gag RNA and comparing it to the initial time point 4 h after exposure (Fig. S1A). We found that the protein levels of CYR61 and LPAR1 were increased in HIV-infected PCLS compared to the uninfected control exposed to 293T supernatant. In addition, we also detected a reduction of pYAP protein levels in a time-dependent manner post HIV infection (Fig. S1B). Minimal tissue degradation of PCLS were observed over 96 h in a viability assay (Fig. S1C).

Using the PCLS model, we studied the profibrotic effects of HIV in human liver tissue. HIV infection/exposure for 72 h significantly increased *COL1A1*, *αSMA*, *TIMP1*, and *CTGF* mRNA and CYR61, LPA, LPAR, pAKT, and AKT protein levels within PCLS compared to slices not exposed to the virus (Fig. 2D,E). In addition, we detected a reduction of pYAP protein levels after HIV infection/exposure with no effect on total YAP levels (Fig. 2E). TGFβ treatment was used as a positive control to induce a fibrotic program in PCLS. These findings suggest that HIV infection/exposure in healthy PCLS is associated with an elevation of fibrogenesis markers and coincides with a decrease in YAP activation.

HIV induced the YAP-dependent upregulation of YAP-responsive genes among Huh7 cells and a 3D liver model

Having recapitulated YAP activation by HIV in studies using our humanized mouse model, *ex vivo* pHHs, and in PCLS, we next evaluated a YAP knockout variant (H7Y5)[11] to further analyze YAP regulation. As described in our previous work, the H7Y5 cell line was created via lentiviral vectors expressing short-hairpin RNAs (shRNA) targeting YAP (shY5). Huh7 cells were also transduced with either a control vector expressing a non-targeting control shRNA (NTshControl) or with shY5 (TRCN0000107265). YAP knockdown was confirmed by western blot.[11] As expected, HIV exposure induced *ANKRD*, *CYR61*, and *CTGF* mRNA expression in NTshControl cells. We did not detect any alteration in mRNA levels of YAP-dependent genes post HIV infection in H7Y5 cells (Fig. 3A). Furthermore, we detected an induction of CYR61 protein, but not in total YAP, after exposure to HIV in NTshControl cells. Levels of pYAP were reduced significantly post HIV exposure. Predictably, there was less YAP and YAP-related protein expression in the H7Y5 cell line (Fig. 3B). Overall, HIV exposure had no significant effect on the induction of YAP activation in the H7Y5 cell line (Fig. 3A,B). Additionally, YAP nuclear immunostaining was enhanced in HIV-exposed NTshControl cells compared to 293T(mock)-infected Huh7 or HIV-exposed H7Y5 cells (Fig. 3C).

In order to further investigate the relationship between HIV and YAP *in vitro* as well as the important role of cell-cell communication, we utilized a 3D spheroid model which has been described in our previous work.[11] This model allows for the co-culturing of hepatocytes and HSCs, thereby more accurately replicating the complex liver milieu. In spheroids with a core of Huh7 cells enveloped by primary HSCs (pHSCs), we found that HIV was associated with elevated αSMA, TIMP1, COL1A1, and COL3A1 mRNA and protein levels within exposed spherocytes compared to the unexposed control. This upregulation was suppressed by YAP silencing in spheroids with a core of H7Y5 cells enveloped by pHSC cells (Fig. 3D,E). This *in vitro* 3D mechanistic study provides clear evidence that hepatocyte YAP is necessary for HSC activation, and by extension fibrosis progression, in HIV-related liver disease.

HIV protein gp120 induced the upregulation of YAP-related genes and the expression of profibrotic markers in a YAP-dependent fashion

To test whether individual HIV proteins can induce YAP activation in hepatocytes, we incubated a panel of purified recombinant HIV-1 proteins, including gp120, Gag, and Rev with Huh7 cells. We consistently found a greater than 2-fold increase in *CTGF*, *CYR61*, and *ANKRD* (2-, 4-, and 6-fold changes in mRNA level, respectively) after gp120 treatment compared to DMSO-treated Huh7 cells (p <0.005). Other HIV proteins had little to no effect on YAP-related genes (Fig. 4A). gp120 treatment also induced CYR61 and reduced pYAP protein expression after 72 h compared with DMSO, Gag, and Rev-treated Huh7 cells (Fig. 4B). To evaluate the impact of gp120 treatment on HSC activation, we collected conditioned media (CM) from Huh7 cells treated with gp120 for 72 h and incubated the CM with pHSCs for 48 h (Fig. 4C,D). The activation of HSCs was more profound following exposure to CM collected from Huh7 cells treated with gp120 compared to control CM harvested from DMSO-treated Huh7 cells. HSC activation significantly increased mRNA and protein levels of the profibrotic markers α-SMA, COL1A1, and COL3A1 in comparison to pHSCs treated with Huh7/DMSO CM for 48 h. Additionally, we detected YAP activation in HSCs via significant induction of *CTGF* and *CYR61* mRNA levels, induction of CYR61 protein, and a reduction of pYAP protein expression. There was no change in total YAP protein expression. Treating pHSCs with gp120 directly had little to no effect on pHSC activation, demonstrating that residual gp120 in the CM has no direct effect on pHSC activation (Fig. S2). These data suggest that HIV-gp120 alone can promote hepatocyte YAP activation in hepatocytes and promote subsequent pHSC activation.

LPA, PI3K, and AKT regulate HIV-induced YAP activation in Huh7 cells

Current evidence implicates LPA and its receptors (LPARs) as upstream regulators of the YAP pathway.[18,20] Initially, we

(Mann-Whitney *U* test). (C) Representative immunoblot of YAP-related pathways and the proposed LPA/YAP axis within pHHs exposed to HIV for 72 h. Data presented as mean relative signal strength generated via computer analysis. pYAP and YAP are presented as the proportion of total YAP that has been phosphorylated. Levels of significance: $p_{(CYR61)}$ <0.001, $p_{(AKT)}$ <0.001, $p_{(pAKT)}$ <0.001, $p_{(pYAP/YAP)}$ = 0.087 (ANOVA). (D, E) qRT-PCR and immunoblot analysis of YAP-related gene expression and profibrotic markers in PLCS exposed to 293T supernatant (mock) or HIV for 72 h. TGFβ-treated cells were used as a positive control for activated HSCs. Levels of significance: $p_{(COL1A1/HIV)}$ = 0.012, $p_{(COL1A1/TGFβ)}$ = 0.028, $p_{(αSMA/HIV)}$ = 0.700, $p_{(αSMA\ /TGFβ)}$ = 0.017, $p_{(TIMP1/HIV)}$ = 0.006, $p_{(TIMP1/TGFβ)}$ = 0.006, $p_{(CTGF/HIV)}$ = 0.010, $p_{(CTGF/TGFβ)}$ = 0.006 (Mann-Whitney *U* test). Data are shown as mean ± SEM (n = 3 biological replicates). pHHs, primary human hepatocytes; PCLS, precision cut liver slice; qRT-PCR, quantitative reverse-transcription PCR.

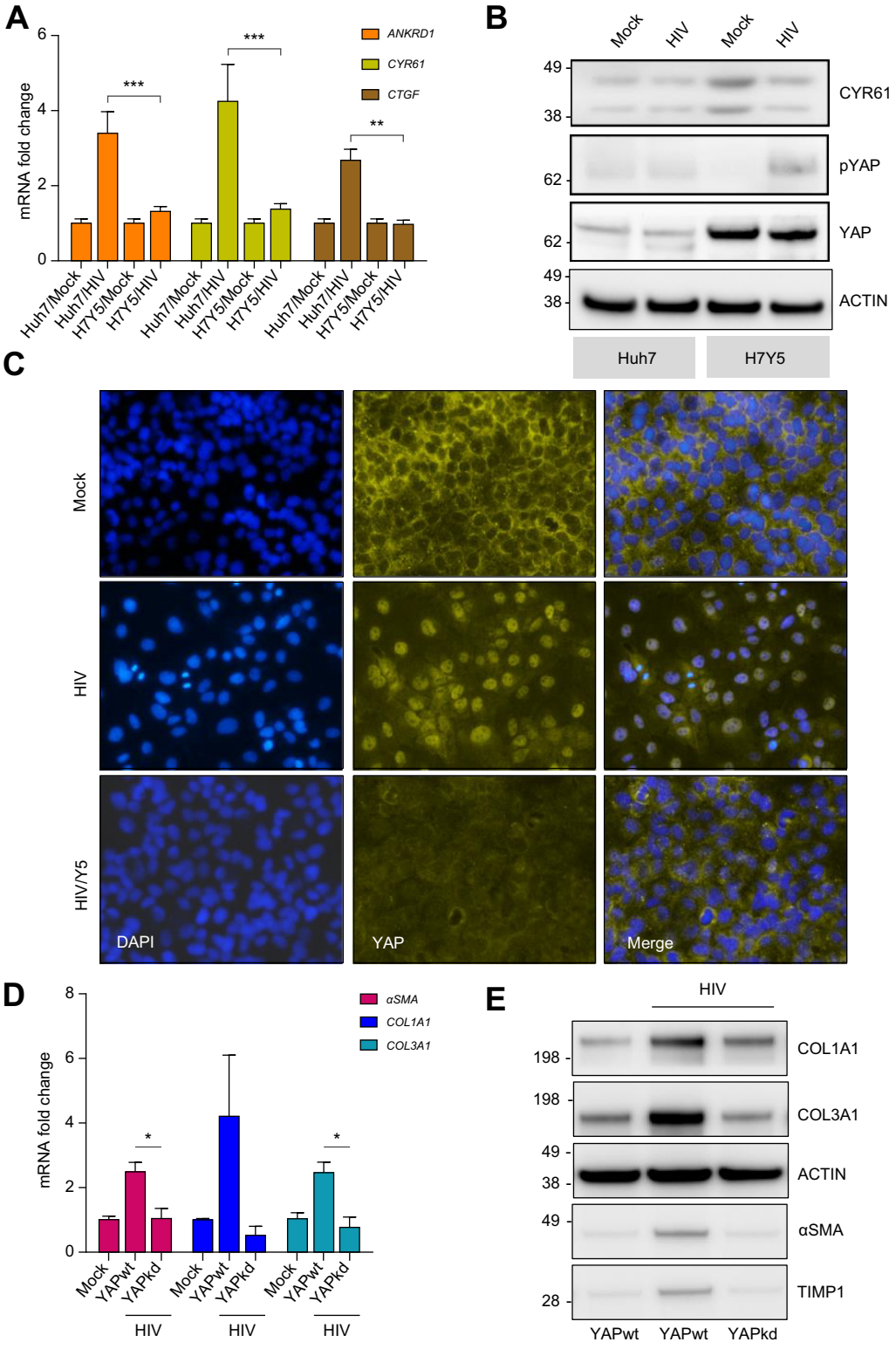

Fig. 3. HIV induced the upregulation of YAP-related genes in a YAP-dependent fashion among Huh7 cells and in a 3D liver model. (A) Huh7 cells transduced with a non-targeting shRNA vector (NTshControl) and a YAP-knockdown cell line (H7Y5) were exposed to mock (unmodified media) or HIV for 72 h. YAP-related gene expression was measured by qRT-PCR. Levels of significance: $p_{(ANKRD)}$ <0.001, $p_{(CYR61)}$ <0.001, $p_{(CTGF)}$ = 0.003 (ANOVA). (B) Western blot analysis of YAP-related pathways in NTshControl cells and H7Y5 cells exposed to HIV for 72 h. (C) YAP (CY5, yellow) nuclear translocation within of NTshControl cells (un)exposed to HIV and H7Y5 cells exposed to HIV for 72 h. Images are presented at 20X magnification. (D) Spheroids comprised of Huh7 (YAPwt) or H7Y5 (YAPkd) cells surrounded by pHSCs were exposed to HIV for 72 h. After harvesting the entire spheroid, fibrogenic related genes were analyzed using qRT-PCR. Levels of significance: $p_{(\alpha SMA)}$ = 0.010, $p_{(COL1A1)}$ = 0.126, $p_{(COL3A1)}$ = 0.031 (Student's *t* test). (E) Representative immunoblot of characteristic fibrogenic pathways in YAPwt and YAPkd spheroids. mRNA values were normalized to GAPDH. Data are shown as mean ± SEM (n = 3 biological replicates; **p* <0.05, ***p* <0.01, ****p* <0.001). qRT-PCR, quantitative reverse-transcription PCR; shRNA, short-hairpin RNA.

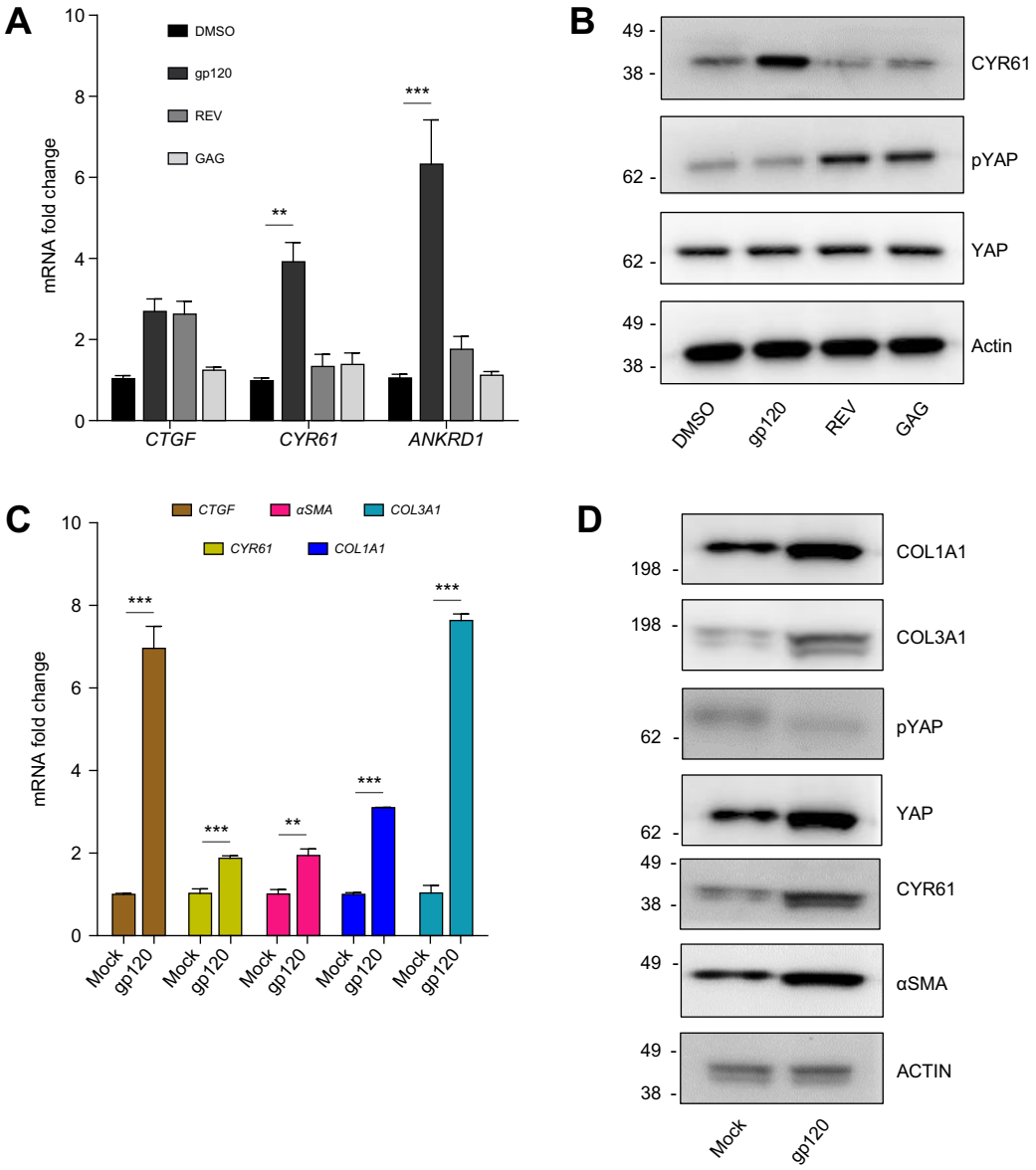

Fig. 4. HIV protein gp120 induces the upregulation of YAP-related genes and the expression of profibrotic markers in a YAP-dependent fashion. (A, B) Huh7 cells were exposed to mock (DMSO) or various HIV proteins (gp120, REV, and GAG) at 10 μg/ml for 72 h. Levels of significance: $p_{(CTGF/gp120)}$ = 0.057, $p_{(CTGF/REV)}$ = 0.098, $p_{(CTGF/GAG)}$ = 0.859, $p_{(CYR61/gp120)}$ = 0.001, $p_{(CYR61/REV)}$ = 0.711, $p_{(CYR61/GAG)}$ = 0.725, $p_{(ANKRD/gp120)}$ <0.001, $p_{(ANKRD/REV)}$ = 0.461, $p_{(ANKRD/GAG)}$ = 0.955 (ANOVA). (C, D) pHSCs were cultured with conditioned media from Huh7 cells exposed to mock (DMSO) or gp120. mRNA fold change and protein levels were measured for YAP-related genes and HSC activation markers and compared with unexposed cells. mRNA values were normalized to GAPDH. Levels of significance: $p_{(CTGF)}$ <0.001, $p_{(CYR61)}$ <0.001, $p_{(αSMA)}$ = 0.008, $p_{(COL1A1)}$ <0.001, $p_{(COL3A1)}$ <0.001 (Student's *t* test). Data are shown as mean ± SEM are graphed (n = 3 biological replicates; **p* <0.05, ***p* <0.01, ****p* <0.001).

sought to determine whether HIV infection impacted LPAR expression within Huh7 cells. Under typical conditions, we detected mRNA expression of each LPAR in hepatocytes (Huh7), except for LPAR4. LPAR4 is canonically not expressed in hepatocytes, but LPAR1, LPAR3, and LPAR6 have been linked to liver fibrosis and HCC.[39–41] LPAR1 antagonism is known to reduce steatosis in murine and human hepatocytes via downregulation of CD36.[42] In the context of HIV exposure, there was a significant induction of LPAR1 expression. LPAR6 was also induced; however, the changes in both mRNA and protein levels were not significant compared to mock (293T supernatant) treated cells (Fig. 5A,B). HIV exposure did not alter the mRNA expression of *LPAR2*, *LPAR3*, or *LPAR5*. Interestingly, by treating Huh7 cells with LPA (10 μM) for 4 h, we induced an upregulation (1.5-4 fold) of multiple YAP-related genes, including *CYR61* and *ANKRD*. No changes in *YAP* expression were detected (Fig. 5C) in comparison with DMSO-treated cells. To further investigate the precise mechanism by which HIV activates YAP, Huh7 cells were treated for 72 h with chemical inhibitors of well-known upstream YAP regulators: ERK (LY3214996 5 μM), LPA (Ki16425 10 μM), PI3K (LY294002 20 μM) and AKT (afuresertib 10 μM). Cells in the mock treatment were exposed solely to DMSO. Pretreatment with LPA, PI3K, and AKT inhibitors significantly reduced YAP activation

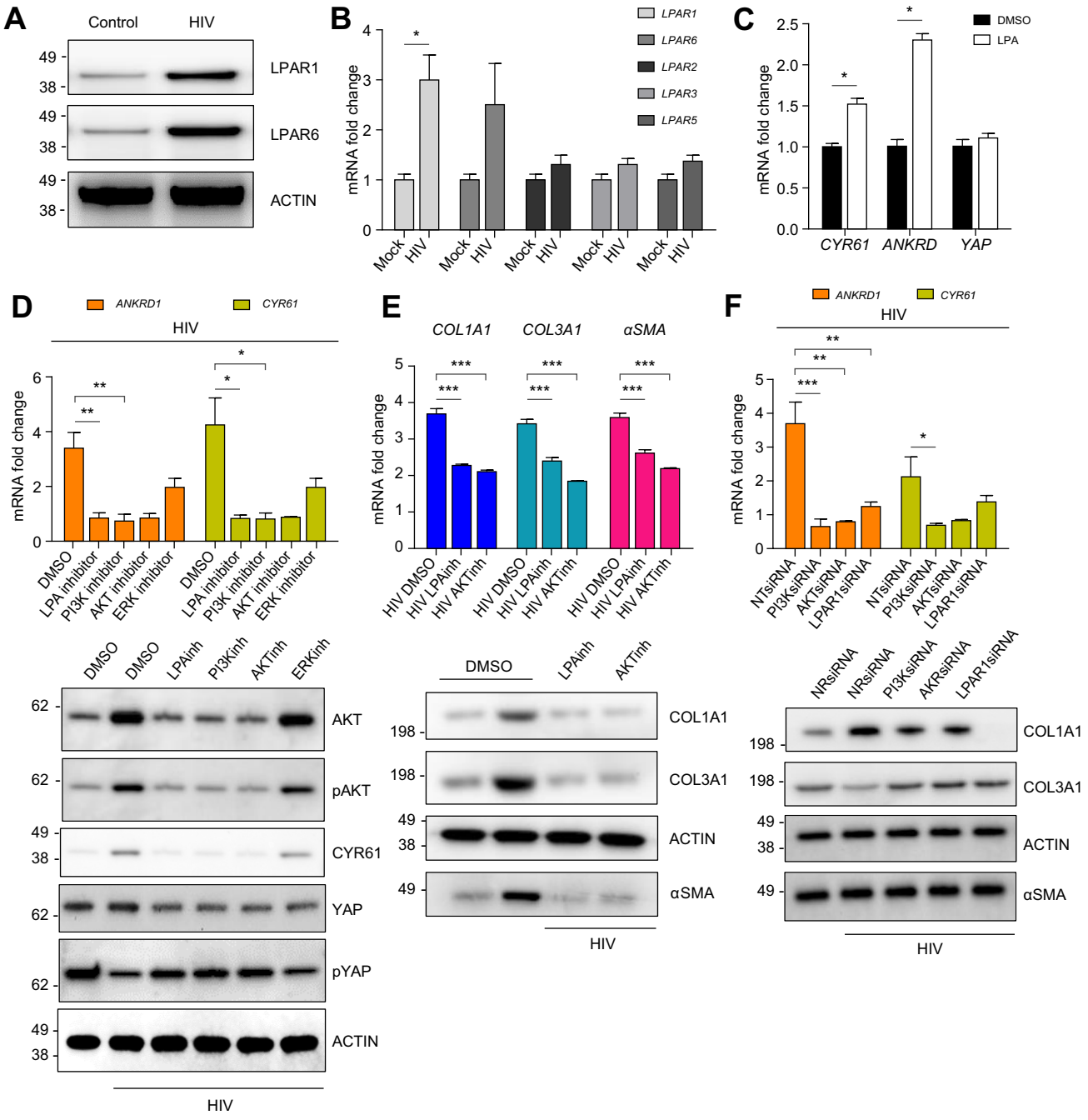

Fig. 5. HIV upregulates YAP-related genes via a pathway that includes LPAR1, AKT, and PI3K. This pathway is dependent upon hepatocyte derived YAP. (A, B) mRNA expression of LPA receptors and representative protein expression of LPAR1 and LPAR6 from three independent experiments (n = 3) in Huh7 cells exposed to HIV for 3 days comparison to unexposed cells. Levels of significance: $p_{(LPAR1)}$ = 0.024, $p_{(LPAR6)}$ = 0.057, $p_{(LPAR2)}$ = 0.267, $p_{(LPAR3)}$ = 0.167, $p_{(LPAR5)}$ = 0.200 (Mann-Whitney *U* test). (C) YAP-related gene mRNA fold change in Huh7 cells post-LPA treatment for 4 h in comparison to unexposed cells. Levels of significance: $p_{(CYR61)}$ = 0.028, $p_{(ANKRD)}$ = 0.028, $p_{(YAP)}$ = 0.576 (Mann-Whitney *U* test). (D) Huh7 cells were treated with mock (DMSO), LPA, PI3K, AKT, and ERK inhibitors and exposed to HIV for 3 days post inhibitor exposure. The mRNA fold changes were analyzed in YAP-related genes *ANKRD1* and *CYR61* in comparison with untreated Huh7 cells. Protein levels were measured for CYR61, (p)AKT, and (p)YAP. Levels of significance: $p_{(ANKRD/LPA)}$ = 0.003, $p_{(ANKRD/PI3K)}$ = 0.008, $p_{(ANKRD/AKT)}$ = 0.857, $p_{(ANKRD/ERK)}$ = 0.111, $p_{(CYR61/LPA)}$ = 0.017, $p_{(CYR61/PI3K)}$ = 0.024, $p_{(CYR61/AKT)}$ = 0.229, $p_{(CYR61/ERK)}$ = 0.114 (Mann-Whitney *U* test). (E) pHSCs were exposed to the conditioned media of Huh7 cells treated with LPA and AKT inhibitors for 2 days. mRNA fold change and protein levels were measured for fibrosis-related pathways and pHSC activation markers. $p_{(COL1A1/LPA)}$ <0.001, $p_{(COL1A1/AKT)}$ <0.001, $p_{(COL3A1/LPA)}$ = <0.001, $p_{(COL3A1/AKT)}$ <0.001, $p_{(\alpha SMA\ /LPA)}$ <0.001, $p_{(\alpha SMA\ /AKT)}$ <0.001 (ANOVA). (F) siRNA targeting PI3K, AKT, and LPA were transfected into Huh7 cells and exposed to HIV for 3 days. qRT-PCR analysis measured *ANKRD1* and *CYR61* mRNA expression, while western blot measured CYR91 and (p)YAP protein levels. All mRNA values were normalized to *GAPDH*. Levels of significance: $p_{(ANKRD/PI3K)}$ <0.001, $p_{(ANKRD/AKT)}$ = 0.001, $p_{(ANKRD/LPAR1)}$ = 0.001, $p_{(CYR61/PI3K)}$ = 0.034, $p_{(CYR61/AKT)}$ = 0.051, $p_{(CYR61/LPAR1)}$ = 0.246 (ANOVA). Data are presented as mean ± SEM (n = 3 biological replicates).

after HIV exposure. The reduction in YAP activation was mediated through the induction of pYAP in addition to the reduction of CYR61, AKT, and pAKT protein levels (Fig. 5D). In contrast, the ERK1 and 2 inhibitor failed to alter YAP activation after HIV exposure (Fig. 5D).

To evaluate the impact of treating hepatocytes with either an LPAR (Ki16425) or AKT (afuresertib) inhibitor on HSC activation, we performed pHSC CM experiments. Conditioned media from Huh7 cells treated with either LPA or AKT inhibitors or DMSO for 72 h (Fig. 5D) were harvested and incubated with pHSCs for 48 h. HSC activation was significantly reduced after treatment with CM from HIV-exposed inhibitor-treated Huh7 cells compared to treatment with CM from HIV-exposed inhibitor-naïve Huh7 cells. This reduction of HSC activation was measured via COL1A1, COL3A1, and αSMA mRNA and protein levels (Fig. 5E). To further assess this pathway, we independently depleted PI3K, AKT, and LPAR1 expressed in the Huh7 cells by small interfering (si)RNA and then assessed the expression of YAP-regulated genes in Huh7 cells. Gene knockdown was confirmed by immunoblot (Fig. S3). Silencing of PI3K and AKT had the greatest effect in suppressing *ANKRD1* and *CYR61* mRNA expression (2-3-fold reduction; $p < 0.05$) following HIV exposure (Fig. 5F). Furthermore, PI3K, AKT, and LPAR1 small-interfering RNA (siRNA) transfection increased YAP phosphorylation and reduced CYR61 protein expression in HIV-exposed Huh7 cells (Fig. 5F). In summary, these data indicate that LPA, PI3K, and AKT are all critical regulators of YAP activation in the context of HIV.

Discussion

Since the onset of the HIV epidemic, our understanding of chronic liver diseases in PLWH has grown significantly. Despite this dramatic change in knowledge, liver disease remains a leading cause of non-AIDS-related mortality among PLWH, and current suppressive ART does not fully ameliorate liver disease progression. This study reveals that HIV infection produces liver fibrosis through activation of the YAP pathway. Moreover, we show that inhibition or knock-down of the upstream LPA, PI3K, and AKT pathways reverse HIV-induced HSC activation and liver fibrosis.

In PLWH, HIV-induced gut barrier disruption and microbial translocation[43–45] may alter the expression of various molecules produced by the liver, leading to YAP activation in hepatocytes. *In vitro* models may not fully recapitulate liver fibrosis induced through HIV-related YAP signaling. The traditional 2D and 3D liver models used in this study rely, at least partially, on transformed cell lines, modifying cellular responses and predisposing them to maladaptive phenotypes compared to mature human cells. For instance, hepatocytes dedifferentiate in response to injury and have been shown to express elevated levels of CXCR4, suggesting that the effects of HIV would be more pronounced in the Huh7 cell line or in PLWH with underlying conditions.[46] This issue was mitigated in our cell line work by the inclusion of pHHs. Notably, the robust humanized mouse model reported previously[35,36,47,48] and used in this work is the most authentic model for liver function *in vivo*, particularly as it relates to HIV infection. A salient observation from this model was the drastic rise in total Yap protein levels after HIV infection. Yap upregulation is well documented in murine fibrosis models including carbon tetrachloride treatment and HCC.[49,53] In addition, YAP expression is positively correlated with liver fibrosis stage in humans with metabolic dysfunction-associated steatotic liver disease. This link is mediated by Kupffer cells releasing pro-inflammatory cytokines which activate HSCs and contribute to fibrogenesis.[54] HIV infection produces various cellular stressors which damage the liver and promote HSC activation and immune cell infiltration.[50] This environment presumably alters the YAP activation and expression profile of many cell types. As a result, harvesting the lysate of whole livers likely masked the reduction in the pYap to Yap ratio and ultimately the effects of Yap activation in hepatocytes. Additionally, the antibodies used in the western blot of humanized mice recognize both human and murine proteins, unlike the primers used during qRT-PCR, which may explain the variation in Cyr61 signal strengths between the two assays (Fig. 1B). Critically, HIV treatment still resulted in the nuclear localization of Yap within these humanized mice (Fig. 1C). This was true for both hepatocytes and non-parenchymal cells. In addition to the direct effects of immune cell infiltration on the liver, inflammatory cytokines circulating during viral infection promote YAP activation within the immune cells themselves.[54] Further study on the role of cellular cross talk on YAP activation, and its origin in liver cell populations, is warranted. Nevertheless, having only one *in vivo* model represents a limitation of this study. Taken together, our models provide a clearer and more comprehensive picture of liver fibrogenesis.

There has been increased work related to hepatic fibrosis in recent years. Studies have reported evidence that LPA and autotaxin (ATX) are necessary for the development of liver fibrosis and HCC.[51] Elevated LPA levels have been correlated with fibrosis stage in both humans and rats infected with HCV.[25,26] LPA signaling is now a known regulator of fibrogenesis in both HCC and cholangiocarcinoma,[27] and a recent study found that serum LPA concentrations are elevated in HIV-infected patients.[28] All these studies contextualize the findings of our work, and cumulatively, they suggest that LPA is a relevant agent in the pathological development of liver fibrosis in PLWH. This study explored the possibility that LPA acts as a fibrosis-inducing agent in PLWH and determined that the effects of several components in the LPA/LPAR1/YAP axis are critical to the fibrotic response to HIV infection.

ATX is the enzyme that hydrolyzes lysophosphatidylcholine into LPA through its unique lysophospholipase D activity, thereby regulating LPA.[52] In the context of hepatic fibrosis, the altered functional capacity appears to reduce the clearance of ATX.[53,54] As a result, ATX has proven to be a reliable biomarker of liver fibrosis.[55] However, despite this known correlation between ATX and hepatic fibrosis, we did not observe induction of ATX in hepatocytes following HIV exposure. One explanation may be that ATX and LPA may derive from other liver cells, such as macrophages[56] (the main target of HIV infection in the liver) and HSCs (critical contributors to extracellular matrix synthesis). It is also important to consider that LPA is produced by HIV-infected cells systemically and is then carried to the liver via the circulatory system. The relationship between ATX and hepatic fibrogenesis among PLWH remains a target for further research.

LPA is a lipid factor that can trigger diverse responses in the liver; to evaluate the effect of LPA as a profibrotic factor and its

dependence on YAP activation, we used as a readout the profibrotic factor CTGF, which is overexpressed in several chronic diseases.[16] We previously demonstrated that CTGF/YAP activation is induced in metabolic dysfunction-associated steatotic liver disease and steatohepatitis,[11] while hepatic YAP knockout reduced liver fibrosis in mice fed a CDAHFD (choline-deficient, L-amino acid-defined, high-fat diet)[11] that reproducibly models metabolic dysfunction-associated steatohepatitis. We found that the CDAHFD upregulated YAP activation as well as CTGF mRNA and protein levels, in accordance with the severity of fibrosis expressed by induction of COL1A1 and TIMP1 mRNA and protein levels.[11] In a recent study, we investigated whether the fibrotic and YAP-related response to HIV could be prevented by interfering with LPA signaling pathways via the well-known LPAR1 and LPAR3 inhibitor, Ki16425. We found that Ki16425 not only abrogated the activation of YAP in hepatocytes, but also prevented signaling which promotes the production of extracellular matrix proteins in pHSC (Fig. 5D,E). Considering the induction of LPAR1 and LPAR6 expression post-HIV exposure in hepatocytes (Fig. 5A,B), we also sought to evaluate the effect of *LPAR1* and *LPAR6* siRNA on HIV's ability to activate YAP and induce a profibrotic program. Our results indicate that *LPAR1* knock-down reduces YAP activation and the fibrotic response to HIV exposure (Fig. 5F). *LPAR6* silencing by siRNA has only a minor effect on YAP activation in Huh7 cells after exposure to HIV. To further explore the downstream contributors in the LPA/YAP pathway, we also utilized inhibitors and siRNA for signaling pathways typically activated by LPA (such as PI3K, ERK1/2, AKT, and YAP) in different cell types.[57,58] PI3K and AKT reduced YAP activation in hepatocytes post-HIV exposure, as opposed to ERK1/2 (Fig. 5D). In the same fashion as LPA, CM from hepatocytes treated with AKT inhibitors prevented the activation of pHSC. We also found that Huh7 cells treated for only 4 h with LPA had significantly increased activation of the YAP pathway (Fig. 5C). This effect was not observed in pHSCs.

Our data reveals a novel mechanism linking liver fibrosis in PLWH to the LPA/LPAR1/YAP pathway. This mechanism has primarily been identified within hepatocytes and in their interactions with HSCs, however important questions about the role of YAP activation in other hepatic cell types remain. Our PCLS and humanized mouse models have demonstrated that YAP is induced broadly across a general hepatic fibrosis environment as well. Further characterization of the YAP profile within macrophages, among other cell types, and their contributions to hepatic fibrogenesis warrant further study. Exploring the details of the Hippo pathway will be critical to elucidating the pathogenesis of liver damage in PLWH. These findings also suggest a possible therapeutic role for LPA axis inhibitors in HIV-related liver fibrosis.

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Abbreviations

ART, antiretroviral therapy; ATX, autotaxin; CM, conditioned media; CTGF, connective tissue growth factor; MST1/2, mammalian Ste20-like kinases 1/2; LATS1/2, large tumor suppressor 1/2: pHH, primary human hepatocyte; (p)HSC, (primary) hepatic stellate cell; hu-mice, humanized mice: LPA, lysophosphatidic acid; LPARs, lysophosphatidic acid receptors; PCLS, precision cut liver slices; PLWH, people living with HIV; qRT-PCR, quantitative reverse-transcription PCR; shRNA, short-hairpin RNA; siRNA, small-interfering RNA; YAP, Yes-associated protein 1.

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Conflict of interest

The authors have nothing to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

S.S. lead conceptualization, design, funding/resource acquisition, data collection, formal analysis, and drafting/editing the manuscript; R.C. lead resource acquisition and supervision while contributing towards formal analysis and editing the manuscript; V.A.S. contributed towards conceptualization, design, funding acquisition, data collection, formal analysis, and drafting/editing the manuscript; B.A.F. and A.J.J. contributed towards data collection and editing the manuscript; Y.W., S.K.K., M.X., W.L., and N.A. contributed towards data collection and formal analysis; J.A. and L.S. contributed resources for this study.

Data availability statement

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2024.101163.

References

[1] Bosh KA, Hall HI, Eastham L, et al. Estimated annual number of HIV infections horizontal line United States, 1981-2019. MMWR Morb Mortal Wkly Rep 2021;70:801–806.

[2] Falade-Nwulia O, Thio CL. Liver disease, HIV and aging. Sex Health 2011;8:512–520.

[3] Sherman KE, Peters MG, Thomas DL. HIV and the liver. Top Antivir Med 2019;27:101–110.

[4] Chamroonkul N, Bansal MB. HIV and the liver. Nat Rev Gastroenterol Hepatol 2019;16:1–2.

[5] Zhang L, Bansal MB. Role of kupffer cells in driving hepatic inflammation and fibrosis in HIV infection. Front Immunol 2020;11:1086.

[6] Nomiyama H, Hieshima K, Nakayama T, et al. Human CC chemokine liver-expressed chemokine/CCL16 is a functional ligand for CCR1, CCR2 and CCR5, and constitutively expressed by hepatocytes. Int Immunol 2001;13:1021–1029.

[7] Ganesan M, Poluektova LY, Kharbanda KK, et al. Human immunodeficiency virus and hepatotropic viruses co-morbidities as the inducers of liver injury progression. World J Gastroenterol 2019;25:398–410.

[8] Tuyama AC, Hong F, Saiman Y, et al. Human immunodeficiency virus (HIV)-1 infects human hepatic stellate cells and promotes collagen I and monocyte chemoattractant protein-1 expression: implications for the pathogenesis of HIV/hepatitis C virus-induced liver fibrosis. Hepatology 2010;52:612–622.

[9] Francois F, Klotman ME. Phosphatidylinositol 3-kinase regulates human immunodeficiency virus type 1 replication following viral entry in primary CD4+ T lymphocytes and macrophages. J Virol 2003;77:2539–2549.

[10] Hamada K, Maeda Y, Mizutani A, et al. The phosphatidylinositol 3-kinase p110alpha/PTEN signaling pathway is crucial for HIV-1 entry. Biol Pharm Bull 2019;42:130–138.

[11] Salloum S, Jeyarajan AJ, Kruger AJ, et al. Fatty acids activate the transcriptional coactivator YAP1 to promote liver fibrosis via p38 mitogen-activated protein kinase. Cell Mol Gastroenterol Hepatol 2021;12:1297–1310.

[12] Noguchi S, Saito A, Nagase T. YAP/TAZ signaling as a molecular link between fibrosis and cancer. Int J Mol Sci 2018;19.
[13] Piersma B, Bank RA, Boersema M. Signaling in fibrosis: TGF-beta, WNT, and YAP/TAZ converge. Front Med (Lausanne) 2015;2:59.
[14] Gokey JJ, Patel SD, Kropski JA. The role of hippo/YAP signaling in alveolar repair and pulmonary fibrosis. Front Med (Lausanne) 2021;8:752316.
[15] Li W, Xiao J, Zhou X, et al. STK4 regulates TLR pathways and protects against chronic inflammation-related hepatocellular carcinoma. J Clin Invest 2015;125:4239–4254.
[16] Ramazani Y, Knops N, Elmonem MA, et al. Connective tissue growth factor (CTGF) from basics to clinics. Matrix Biol 2018;68-69:44–66.
[17] Nguyen-Lefebvre AT, Selzner N, Wrana JL, et al. The hippo pathway: a master regulator of liver metabolism, regeneration, and disease. FASEB J 2021;35:e21570.
[18] Yu FX, Zhao B, Panupinthu N, et al. Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. Cell 2012;150:780–791.
[19] Piccolo S, Dupont S, Cordenonsi M. The biology of YAP/TAZ: hippo signaling and beyond. Physiol Rev 2014;94:1287–1312.
[20] Riaz A, Huang Y, Johansson S. G-Protein-Coupled lysophosphatidic acid receptors and their regulation of AKT signaling. Int J Mol Sci 2016;17:215.
[21] Avruch J, Zhou D, Fitamant J, et al. Protein kinases of the Hippo pathway: regulation and substrates. Semin Cell Dev Biol 2012;23:770–784.
[22] Zhao Y, Montminy T, Azad T, et al. PI3K positively regulates YAP and TAZ in mammary tumorigenesis through multiple signaling pathways. Mol Cancer Res 2018;16:1046–1058.
[23] Ahmed AA, Abedalthagafi M, Anwar AE, et al. Akt and hippo pathways in ewing's sarcoma tumors and their prognostic significance. J Cancer 2015;6:1005–1010.
[24] Chugh P, Bradel-Tretheway B, Monteiro-Filho CM, et al. Akt inhibitors as an HIV-1 infected macrophage-specific anti-viral therapy. Retrovirology 2008;5:11.
[25] Watanabe N, Ikeda H, Nakamura K, et al. Both plasma lysophosphatidic acid and serum autotaxin levels are increased in chronic hepatitis C. J Clin Gastroenterol 2007;41:616–623.
[26] Watanabe N, Ikeda H, Nakamura K, et al. Plasma lysophosphatidic acid level and serum autotaxin activity are increased in liver injury in rats in relation to its severity. Life Sci 2007;81:1009–1015.
[27] Kaffe E, Magkrioti C, Aidinis V. Deregulated lysophosphatidic acid metabolism and signaling in liver cancer. Cancers (Basel) 2019;11.
[28] Kostadinova L, Shive CL, Judge C, et al. During hepatitis C virus (HCV) infection and HCV-HIV coinfection, an elevated plasma level of autotaxin is associated with lysophosphatidic acid and markers of immune activation that normalize during interferon-free HCV therapy. J Infect Dis 2016;214:1438–1448.
[29] Alkhatib G, Combadiere C, Broder CC, et al. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996;272:1955–1958.
[30] Choe H, Farzan M, Sun Y, et al. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 1996;85:1135–1148.
[31] Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. Nature 1996;381:661–666.
[32] Doranz BJ, Rucker J, Yi Y, et al. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 1996;85:1149–1158.
[33] Dragic T, Litwin V, Allaway GP, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature 1996;381:667–673.
[34] Ahodantin J, Nio K, Funaki M, et al. Type I interferons and TGF-beta cooperate to induce liver fibrosis during HIV-1 infection under antiretroviral therapy. JCI Insight 2022;7.
[35] Li G, Zhao J, Cheng L, et al. HIV-1 infection depletes human CD34+CD38- hematopoietic progenitor cells via pDC-dependent mechanisms. Plos Pathog 2017;13:e1006505.
[36] Cheng L, Ma J, Li G, et al. Humanized mice engrafted with human HSC only or HSC and thymus support comparable HIV-1 replication, immunopathology, and responses to ART and immune therapy. Front Immunol 2018;9:817.
[37] Kmiec Z. Cooperation of liver cells in health and disease. Adv Anat Embryol Cell Biol 2001;161:1–151. III-XIII.
[38] Nunoya J, Washburn ML, Kovalev GI, et al. Regulatory T cells prevent liver fibrosis during HIV type 1 infection in a humanized mouse model. J Infect Dis 2014;209:1039–1044.
[39] Eraky SM, El-Mesery M, El-Karef A, et al. Silymarin and caffeine combination ameliorates experimentally-induced hepatic fibrosis through down-regulation of LPAR1 expression. Biomed Pharmacother 2018;101:49–57.
[40] Zuckerman V, Sokolov E, Swet JH, et al. Expression and function of lysophosphatidic acid receptors (LPARs) 1 and 3 in human hepatic cancer progenitor cells. Oncotarget 2016;7:2951–2967.
[41] Mazzocca A, Dituri F, De Santis F, et al. Lysophosphatidic acid receptor LPAR6 supports the tumorigenicity of hepatocellular carcinoma. Cancer Res 2015;75:532–543.
[42] Lua I, Balog S, Yanagi A, et al. Loss of lysophosphatidic acid receptor 1 in hepatocytes reduces steatosis via down-regulation of CD36. Prostaglandins Other Lipid Mediat 2021;156:106577.
[43] Zevin AS, McKinnon L, Burgener A, et al. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. Curr Opin HIV AIDS 2016;11:182–190.
[44] Dillon SM, Frank DN, Wilson CC. The gut microbiome and HIV-1 pathogenesis: a two-way street. AIDS 2016;30:2737–2751.
[45] Lujan JA, Rugeles MT, Taborda NA. Contribution of the microbiota to intestinal homeostasis and its role in the pathogenesis of HIV-1 infection. Curr HIV Res 2019;17:13–25.
[46] Aguilar-Bravo B, Arino S, Blaya D, et al. Hepatocyte dedifferentiation profiling in alcohol-related liver disease identifies CXCR4 as a driver of cell reprogramming. J Hepatol 2023;79:728–740.
[47] Li G, Zhang Z, Reszka-Blanco N, et al. Specific activation in vivo of HIV-1 by a bromodomain inhibitor from monocytic cells in humanized mice under antiretroviral therapy. J Virol 2019;93.
[48] Bility MT, Cheng L, Zhang Z, et al. Hepatitis B virus infection and immunopathogenesis in a humanized mouse model: induction of human-specific liver fibrosis and M2-like macrophages. Plos Pathog 2014;10:e1004032.
[49] Verboven E, Moya IM, Sansores-Garcia L, et al. Regeneration defects in yap and taz mutant mouse livers are caused by bile duct disruption and cholestasis. Gastroenterology 2021;160:847–862.
[50] Mooring M, Fowl BH, Lum SZC, et al. Hepatocyte stress increases expression of yes-associated protein and transcriptional coactivator with PDZ-binding motif in hepatocytes to promote parenchymal inflammation and fibrosis. Hepatology 2020;71:1813–1830.
[51] Kaffe E, Katsifa A, Xylourgidis N, et al. Hepatocyte autotaxin expression promotes liver fibrosis and cancer. Hepatology 2017;65:1369–1383.
[52] Song Y, Dilger E, Bell J, et al. Large scale purification and characterization of recombinant human autotaxin/lysophospholipase D from mammalian cells. BMB Rep 2010;43:541–546.
[53] Benesch MG, Ko YM, McMullen TP, et al. Autotaxin in the crosshairs: taking aim at cancer and other inflammatory conditions. FEBS Lett 2014;588:2712–2727.
[54] Jansen S, Andries M, Vekemans K, et al. Rapid clearance of the circulating metastatic factor autotaxin by the scavenger receptors of liver sinusoidal endothelial cells. Cancer Lett 2009;284:216–221.
[55] Honda Y, Imajo K, Kobayashi T, et al. Autotaxin is a valuable biomarker for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. Hepatol Res 2019;49:1136–1146.
[56] Wang Z, Shi W, Tian D, et al. Autotaxin stimulates LPA2 receptor in macrophages and exacerbates dextran sulfate sodium-induced acute colitis. J Mol Med (Berl) 2020;98:1781–1794.
[57] Cabello-Verrugio C, Cordova G, Vial C, et al. Connective tissue growth factor induction by lysophosphatidic acid requires transactivation of transforming growth factor type beta receptors and the JNK pathway. Cell Signal 2011;23:449–457.
[58] Jean-Baptiste G, Yang Z, Khoury C, et al. Lysophosphatidic acid mediates pleiotropic responses in skeletal muscle cells. Biochem Biophys Res Commun 2005;335:1155–1162.

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