Original research

Epigenetic mechanisms and metabolic reprogramming in fibrogenesis: dual targeting of G9a and DNMT1 for the inhibition of liver fibrosis

Marina Barcena-Varela, Hannah Paish, Laura Alvarez, Iker Uriarte, Maria U Latasa, Eva Santamaria, Miriam Recalde, Maria Garate, Alex Clavería, Leticia Colyn, Maria Arecchaderra, Maria J Iraburu, Malgorzata Milkiewicz, Piotr Milkiewicz, Bruno Sangro, Stuart M Robinson, Jeremy French, Ana Pardo-Saganta, Julen Oyarzabal, Felipe Prosper, Krista Rombouts, Fiona Oakley, Jelena Mann, Carmen Berasain, Matias A Avila, Maite G Fernandez-Barrena

ABSTRACT

Objective Hepatic stellate cells (HSC) transdifferentiation into myofibroblasts is central to fibrogenesis. Epigenetic mechanisms, including histone and DNA methylation, play a key role in this process. Concerted action between histone and DNA-methyltransferases like G9a and DNMT1 is a common theme in gene expression regulation. We aimed to study the efficacy of CM272, a first-in-class dual and reversible G9a/DNMT1 inhibitor, in halting fibrogenesis.

Design G9a and DNMT1 were analysed in cirrhotic human livers, mouse models of liver fibrosis and cultured mouse HSC. G9a and DNMT1 expression was knocked down or inhibited with CM272 in human HSC (hHSC), and transcriptomic responses to transforming growth factor-β1 (TGFβ1) were examined. Glycolytic metabolism and mitochondrial function were analysed with Seahorse-XF technology. Genetic expression regulation was analysed by chromatin immunoprecipitation and methylation-specific PCR. Antifibrogenic activity and safety of CM272 were studied in mouse chronic CCl4 administration and bile duct ligation (BDL), and in human precision-cut liver slices (PCLSs) in a new bioreactor technology.

Results G9a and DNMT1 were detected in stromal cells in areas of active fibrosis in human and mouse livers. G9a and DNMT1 expression was induced during mouse HSC activation, and TGFβ1 triggered their chromatin recruitment in hHSC. G9a/DNMT1 knockdown and CM272 inhibited TGFβ1 fibrogenic responses in hHSC. TGFβ1-mediated profibrogenic metabolic reprogramming was abrogated by CM272, which restored gluconeogenic gene expression and mitochondrial function through on-target epigenetic effects. CM272 inhibited fibrogenesis in mice and PCLSs without toxicity.

Conclusions Dual G9a/DNMT1 inhibition by compounds like CM272 may be a novel therapeutic strategy for treating liver fibrosis.

INTRODUCTION

The fibrogenic response is part of the natural reparative reaction in different tissues and organs. This process leads to the formation of a temporary

What is already known on this subject?

► The progression of liver fibrosis depends on the activation and transdifferentiation of hepatic stellate cells (HSC) into a myofibroblastic phenotype.

► Epigenetic mechanisms have been shown to control many aspects of fibrogenesis in the liver.

► Metabolic reprogramming is emerging as a key process in the activation of fibrogenic cells in different organs.

What are the new findings?

► Together with DNMT1, the histone methyltransferase G9a is expressed in fibrogenic cells in cirrhotic human liver, in chronically injured mouse liver and on activation of cultured mouse HSC.

► G9a and DNMT1 expression is required for fibrogenic activation of HSC by transforming growth factor-β1 (TGFβ1).

► Pharmacological targeting of DNMT1 and G9a with the novel first-in-class dual G9a/DNMT1 inhibitor CM272 counteracts the pro-fibrogenic responses and metabolic reprogramming of HSC elicited by TGFβ1.

► CM272 administration shows antifibrogenic activity in clinically relevant mouse models of liver fibrosis and in human precision-cut liver slices without causing toxic effects.

How might it impact on clinical practice in the foreseeable future?

► The development of effective antifibrotic therapies is much needed for chronic liver disease and also for other organs like the lung and kidney. Targeting the complex epigenetic mechanisms involved in fibrogenesis with innovative molecules like CM272 may pave the way for better therapies.
extracellular matrix (ECM) which after wound repair is degraded and tissue architecture is restored. However, when damage persists, as occurs in liver chronic viral infection, alcohol abuse or in non-alcoholic fatty liver disease, the equilibrium between ECM production and removal is ultimately lost resulting in excessive accumulation of a dense ECM rich in fibrillar collagens. This ECM is a physical barrier that perturbs organ’s perfusion, contributes to loss of liver function, progression to cirrhosis and hepatocellular carcinoma development. The pathological relevance of liver fibrogenesis has driven very active research over the past decades. One major finding was the realisation of the highly dynamic nature of the process, including clinical findings showing fibrosis reversion on removal of the causative agent. The major cellular source of collagen are the liver myofibroblasts, mesenchymal cells mainly derived from hepatic stellate cells (HSC) and periportal fibroblasts. In the normal liver, HSC show a quiescent and differentiated phenotype which on hepatic injury is substantially altered. A plethora of cytokines, small molecules and growth factors, with transforming growth factor-β1 (TGFβ1) playing a central role, contribute to HSC activation and conversion into proliferative and inflammatory collagen-secreting myofibroblasts. A profound metabolic reprogramming, including a shift towards aerobic glycolysis, was recently identified as an essential mechanism in HSC activation. Earlier evidence indicated that during fibrosis regression, myofibroblasts were removed by apoptosis or entered a senescent pro-fibrolytic state prone to immune-mediated clearance. However, later studies demonstrated that on cessation of injury, a significant proportion of myofibroblasts also undergo reversion to a deactivated phenotype. Together, these findings attest to the plasticity of HSC and provide valuable insights for the development of much needed antifibrogenic strategies.

Extensive changes in the HSC’s transcriptome occur during their transition into hepatic myofibroblasts and on cessation of injury their reversion to quiescence. In this context, epigenetic mechanisms are increasingly recognised to play a central role. DNA hypomethylation has been associated with fibrogenic gene activation, while repression of genes that maintain HSC differentiation and quiescence was linked to increased methyl-CpG abundance in their regulatory regions. Mechanistically, to control gene expression DNA methylation works in concert with other epigenetic modifications such as acetylation and methylation of lysine residues in histones H3 and H4. The methyl-CpG binding protein MeCP2 plays a key function in this process, orchestrating the activity of the histone methyltransferases (HMTs) enhancer of zeste homolog-2 (EZH2) and absent, small or homeotic disc 1 (ASH1) during the reprogramming of HSC transcriptome to the myofibroblast phenotype. From a translational perspective, it is important to consider that epigenetic modifications are reversible and amenable to pharmacological intervention. Indeed, the antifibrogenic effects of histone deacetylase inhibitors were already reported 20 years ago. More recently, it was demonstrated that targeting DNA-methyltransferases (DNMTs) with 5-azacytidine prevents HSC fibrogenic activation, while inhibition of HMTs halts hepatic fibrosis progression in mice. Similarly, pharmacological inhibition of the H3K9 methyltransferase G9a, also known as euchromatic histone-lysine methyltransferase 2 (EHMT2), has been recently shown to reduce kidney and lung fibrosis, although the underlying mechanisms are not fully understood. Concerted action between DNMTs and HMTs appears a common theme in physiological transcriptional control of tissue homeostasis. However, when dysregulated, this cross-talk can cause disease, including tumourigenesis.

has been demonstrated for G9a, which physically and functionally interacts with DNMT1 driving tumour cell proliferation and adaptation to hypoxia, among other cancer traits. Therefore, the simultaneous targeting of G9a and DNMT methyltransferase activities could be a more effective therapeutic strategy. With this in mind, we recently developed a new series of potent first-in-class, selective and reversible dual small molecule inhibitors against G9a and DNMT activity with an excellent in vivo safety profile. These compounds are very effective against haematological malignancies and also in hepatocellular carcinoma (HCC) models, particularly on HSC-driven HCC growth. Here, we demonstrate the therapeutic potential of dual G9a/DNMT targeting in experimental liver fibrosis and show how this epigenetic mechanism can control TGFβ1-mediated pro-fibrogenic metabolic reprogramming and HSC activation.

**MATERIALS AND METHODS**

**Human samples**

Liver tissue samples and patients’ data were provided by the Biobank of the University of Navarra (Pamplona, Spain) or by the Medical University of Warsaw (Warsaw, Poland). Samples were from patients with HCV (n=10) and HBV (n=10) infection and alcoholic cirrhosis (ALD) (n=10). All of them presented cirrhosis and underwent liver transplantation. Control liver tissue samples (n=5) were obtained from large-margin liver resections of colorectal metastases showing no pathologist-identified microscopic changes of liver disease. Liver tissue specimens were paraffin embedded and stored at −75°C until use. Written informed consent was obtained from each patient.

**Mouse models**

C57BL/6J male mice 6–8 weeks old (n=6–8 mice per group) were used. For acute CCl4 treatment, mice received a 1:1 mixture of CCl4 and olive oil (1 µL CCl4/g body weight, i.p.); 24 hours later, mice received one injection of CM272 (5 mg/kg, i.p.) or vehicle (PBS), and 24 hours later were humanely killed. For chronic CCl4 treatment, mice received a 1:3 mixture of CCl4 and olive oil (0.67 µL CCl4/g body weight, i.p.) twice per week for 6 weeks to induce fibrosis. For the last 2 weeks, animals received daily injections of CM272 (5 mg/kg, i.p.) or PBS. Mice were humanely killed at days 1 and 4 after the last CCl4 injection. Bile duct ligation (BDL) was performed as described. From day 2 post-surgery, animals received daily injections of CM272 (2.5 mg/kg) or PBS (i.p.) and were humanely killed after 11 days.

**Precision-cut liver slices (PCLSs) experiments**

Human liver tissue was obtained from normal resection margins surrounding colorectal metastases from adult patients undergoing surgical resection at the Freeman Hospital (Newcastle upon Tyne, UK). Informed consent was obtained from all patients. PCLSs were obtained from agarose embedded tissues cut with a Leica VT1200S vibratome and cultured in a rocking bioreactor platform as previously described. PCLSs were rested for 24 hours and were then treated with TGFβ1 (3 ng/mL) and platelet-derived growth factor-BB (PDGFB-BB) (50 ng/mL) from Peprotech (London, UK), activin receptor-like kinase-5 inhibitor (Alk5i) SB-525334 (2.5 µM) (Sigma, St. Louis MO, USA) or CM272 (1 µM).

Additional methods are provided in online supplementary methods.
Figure 1  G9A, DNMT1 and α-SMA immunostaining on sections from normal and diseased human and mouse liver tissues. (A) Representative immunostainings showing G9a and DNMT1 detection (arrows) in fibrotic lesions in livers from cirrhotic patients with chronic HCV or HBV infection, or alcoholic liver disease (ALD). α-SMA staining identifies myofibroblasts in association with fibrotic lesions. Images are representative of at least 10 patients per condition. (B) Representative immunostainings showing G9a and DNMT1 detection (arrows) in liver sections from control mice and from animals chronically treated with CCl4 (6 weeks) or 11 days after bile duct ligation (BDL). α-SMA staining identifies myofibroblasts in association with fibrotic lesions. Images are representative of at least six mice per condition.

RESULTS
Expression of G9a and DNMT1 in activated HSC
We performed immunohistochemical staining of liver tissue samples from patients with viral cirrhosis and ALD. We detected the presence of G9a and DNMT1 in activated myofibroblasts (α-smooth muscle actin, α-SMA-expressing cells) (figure 1A, online supplementary figure S1,S2). G9a and DNMT1 were also detected in mouse liver myofibroblasts after chronic CCl4 injury or BDL (figure 1B, online supplementary figure S3). Next, we examined the expression of G9a and DNMT1 in quiescent and...
culture-activated mouse HSC. We found that G9a and DNMT1 protein levels were significantly reduced between day 1 and day 4 of culture in parallel with α-SMA, a marker of HSC myofibroblastic transdifferentiation (figure 2A). The expression of ubiquitin-like with PHD and RING finger domains-1 (UHRF1), a key co-ordinator of DNA methylation during DNA replication and a functional adaptor between DNMT1 and G9a, was also increased in culture-activated HSC (figure 2A). The mRNA levels of these three genes also increased on HSC activation (figure 2A). Interestingly, in LX2 cells, a well-characterised model of human HSC, TGFB1 stimulation induced the rapid recruitment of the three proteins to the nuclear chromatin subfraction, without significantly changing their expression (figure 2B). Combined, these observations suggested a role for G9a and DNMT1, together with UHRF1, in HSC activation. To directly address this point, we examined TGFB1 responses in LX2 cells after siRNA-mediated knockdown of these genes (online supplementary figure S4). We found an overall impairment of TGFB1-activated profibrogenic gene expression, an effect that was particularly strong on G9a downregulation (figure 2C).

Dual targeting of G9a and DNMT1 inhibits hypoxia-driven and TGFB1-driven activation of HSC

These observations and our previous findings suggested that interference with G9a/DNMT1 activities may counteract HSC activation. Therefore, we tested the effects of CM272, our lead G9a/DNMT dual inhibitory compound, on LX2 cells. We demonstrated that CM272 decreased global DNA methylation (5-methyl-cytosine) and H3K9me2 levels without affecting other histone markers (online supplementary figure S5A,B). Next, we observed a marked impairment of TGFB1 effects on key fibrogenic genes expression, including COL1α1 and TGFB1 itself (figure 3A and online supplementary figure S5C), while glial fibrillary acidic protein (GFAP), a marker of quiescent HSC, was upregulated (figure 3A). These effects were reproduced in primary human HSC (online supplementary figure S5D). Interestingly, culture-activation of primary mouse HSC was also reduced by CM272 treatment, as indicated by the expression of Coll1α1, Timp1 and lecithin-retinol acyltransferase (Lrat) (online supplementary figure S5E). In agreement with the impaired response to TGFB1 stimulation when G9a and DNMT1 were knocked down in LX2 cells (figure 2C), we found that combined treatment with the DNMT1 inhibitor decitabine and the G9a inhibitor BIX01294 also dampened the pro-fibrogenic responses to this growth factor (online supplementary figure S5F). Together with TGFB1, hypoxia is considered a major driver of liver fibrogenesis. Consistently, we found that hypoxia stimulated LX2 cells growth and that CM272 inhibited this response as well as basal cell growth under normoxia (figure 3B). Moreover, fibrogenic gene expression induction by hypoxia was also blunted by CM272 (figure 3B). To better understand the effects of CM272 on fibrogenic cells activation, we performed a microarray analysis of gene expression in LX2 cells treated with TGFB1 in the presence or absence of the drug. CM272 markedly affected TGFB1-mediated gene expression regulation, with 1930 upregulated and 1442 downregulated genes (p<0.01) compared with cells treated with TGFB1 alone (figure 3C). Gene ontology (GO) functional classification fundamentally identified categories related to cell growth, differentiation, signalling, metabolism, chromatin regulation and response to hypoxia (figure 3C). Accordingly, when we applied gene set enrichment analysis (GSEA), a significant positive enrichment in genes of the KEGG peroxisome proliferator-activated receptor (PPAR) signalling pathway, as well as the reactome “metabolism of steroid hormones and vitamins A and D”, was detected in cells treated with CM272 (figure 3D). Also consistent with our GO analyses and with the effects of CM272 on TGFB1 and hypoxia-mediated fibrogenic activation, we found significant negative enrichments in gene sets involved in TGFB1, platelet-derived growth factor receptor-β (PDGFRβ) and hypoxia-inducible factor (HIF) pathways (figure 3D). Interestingly, a negative enrichment was also observed in the KEGG glycolysis/gluconeogenesis gene set (figure 3D). Collectively, these findings indicate that G9a/DNMT1 targeting with CM272 profoundly affects the fibrogenic activation of liver myofibroblasts and the involved metabolic adaptations.

Mechanisms of the inhibitory effects of CM272 on hepatic myofibroblasts activation

In view of the antagonism of CM272 on TGFB1 cellular responses, we first checked whether TGFB1 signalling could be affected. We found that CM272 treatment attenuated SMAD3 phosphorylation in response to TGFB1 in LX2 cells (online supplementary figure S6A). Different mechanisms have been involved in the regulation of TGFB1 signalling, among them is the expression of the TGFB1 pseudoreceptor bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI) in different cell types but also in liver myofibroblasts. Interestingly, we observed that CM272 treatment increased the expression of this negative regulator of TGFB1 signalling in LX2 cells (online supplementary figure S6B). However, the antifibrogenic effects of CM272 may extend beyond the direct inhibition of TGFB1 signalling. As previously shown in figure 3A, the basal expression of several genes including TGFB1, PDGFRβ, PAI1, LOX and GFAP was regulated by CM272 in LX2 cells in the absence of TGFB1. Interestingly, we found that these responses were also observed in the presence of the TGFB1 receptor-1 inhibitor (Alk5i) SB-525334 regardless of TGFB1 stimulation (online supplementary figure S6C).

Metabolic reprogramming is emerging as a critical event in fibrogenic activation across different tissue types. Therefore, we examined the effects of CM272 on oxygen consumption rate (OCR; a representation of mitochondrial activity) and the extracellular acidification rate (ECAR; a surrogate for glycolytic rate) in LX2 cells treated with TGFB1. As recently reported, we found that TGFB1 reduced OCR and increased ECAR; however, these effects were attenuated by CM272 (figure 4A). Consistently, the relative contribution to ATP production of glycolysis versus oxidative phosphorylation, which was increased by TGFB1, was mitigated by CM272 treatment (figure 4B). TGFB1-triggered lactate production, a hallmark of the glycolytic phenotype contributing to fibrogenesis, was also attenuated by CM272 (figure 4C). Changes in the expression of key glycolytic and gluconeogenic genes have been mechanistically linked to metabolic reprogramming and activation of fibrogenic cells. Consistently, when glycolysis was inhibited using the glucose analogue 2-deoxy-D-glucose (2DG) (online supplementary figure S7A), we found that TGFB1-mediated fibrogenic gene expression in LX2 cells was impaired (online supplementary figure S7B). Next, we tested the expression of the glycolytic genes hexokinase-1 (HK-1), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), aldolase-A (ALDOA), phosphoglycerate kinase-1 (PGK-1), pyruvate kinase M2 (PKM2) and lactate dehydrogenase A (LDHA) in LX2 cells treated with TGFB1 and CM272. CM272 reduced the basal expression of
Figure 2  Expression and role of G9a, DNMT1 and UHRF1 in liver fibrogenic cells activation. (A) Expression of G9a, DNMT1 and UHRF1 in primary mouse hepatic stellate cells (HSC) during culture activation. Left panel shows a representative Western blot including α-SMA protein levels denoting HSC activation kinetics and Ponceau staining to show equal loading. Right panel shows qPCR analyses of mRNA levels for the indicated genes in the early phase of HSC culture activation. (B) Representative Western blot analyses of G9a, DNMT1 and UHRF1 proteins in the chromatin fraction from nuclear extracts, or total cell lysates, obtained from LX2 cells treated with TGFβ1 for 3 hours. Histone H3 and α-TUBULIN levels are shown to demonstrate equal loading. (C) Influence of G9a, DNMT1 and UHRF1 expression on TGFβ1-mediated fibrosis-related gene expression in LX2 cells. Cells were transfected with G9a, DNMT1 or UHRF1-specific siRNAs, or control siRNAs (siC) and 24 hours later were treated with TGFβ1 for another 24 hours. Graph shows the qPCR analysis of mRNA levels for the indicated genes.
Figure 3  Dual targeting of G9a and DNMT1 inhibits hypoxia-driven and TGFβ1-driven activation of hepatic stellate cells. (A) LX2 cells were treated with CM272 (400 nM) for 24 hours and then stimulated with TGFβ1 (5 ng/mL) for another 24 hours. Expression of fibrogenic activation-related genes and GFAP was evaluated by qPCR. (B) Effect of CM272 on the growth (left panel) and fibrogenic gene expression (right panel) elicited by hypoxia, including transforming growth factor-β1 (TGFβ1), platelet-derived growth factor receptor β (PDGFRβ), tissue inhibitor of metalloproteases 1 (TIMP1), lysyl oxidase (LOX) and lactate dehydrogenase A (LDHA). LX2 cells were treated with CM272 (400 nM) for 24 hours and then grown under normoxic (20% O2) or hypoxic (1% O2) conditions for a further 24 hours. (C) Left panel shows the most relevant GO categories of genes undergoing changes in expression identified by microarray analysis in LX2 cells treated or not with CM272 (400 nM) and then stimulated with TGFβ1 (5 ng/mL) for another 24 hours. Right panel shows a volcano plot displaying differentially expressed genes between LX2 cells treated with TGFβ1 in the presence or absence of CM272. Red dots represent upregulated transcripts and green dots represent transcripts with downregulated expression. (D) Gene set enrichment analysis of microarray gene expression data revealed positive enrichment in gene expression by CM272 in categories related to PPAR signalling and steroid hormone and liposoluble vitamins metabolism, and negative enrichment related to glucose metabolism, hypoxia and fibrogenic activation (TGFβ1 and PDGFRβ pathways).
Figure 4  Dual targeting of G9a and DNMT1 counteracts the pro-fibrogenic metabolic reprogramming of HSC elicited by TGFβ1. (A) Left panel, oxygen consumption rate (OCR) in LX2 cells treated or not with CM272 (400 nM) for 24 hours and then stimulated or not with TGFβ1 (5 ng/mL) for 3 hours. Right panel, extracellular acidification rate (ECAR) in LX2 cells treated as indicated above. (B) Relative ATP production from oxidative phosphorylation (OXPHOS) and glycolysis in LX2 cells treated as indicated above. (C) Lactate production (ie, lactate release to culture medium) by LX2 cells pre-treated or not with CM272 (400 nM) for 24 hours and then stimulated with TGFβ1 (5 ng/mL) for up to 24 hours more. (D) CM272 counteracts the reprogramming of metabolic gene expression elicited by TGFβ1 in LX2 cells. Cells were treated with CM272 (400 nM) for 24 hours and then stimulated with TGFβ1 (5 ng/mL) for another 24 hours as indicated. The expression of genes involved in glycolysis (red letters), the serine–glycine pathway (green letters) and gluconeogenesis (blue letters) was measured by qPCR. (E) Western blot analysis of FBP1 and PGC-1α protein levels in LX2 cells treated with CM272 (200 nM) for 48 hours. Representative blots are shown. (F) Analysis of H3K9me2 levels by qChIP assay in the proximal promoter regions of FBP1 and PGC-1α genes in LX2 cells treated with CM272 (200 nM) for 48 hours. (G) Methylation-specific PCR (MSP) assays of DNA methylation in FBP1 and PGC-1α promoters in control and CM272 (100 nM, 96 hours) treated LX2 cells. Cells were also treated with decitabine (5 µM) as a control for a DNA demethylating agent. Bands in lanes labelled ‘U’ and ‘M’ are PCR products amplified with unmethylation-specific and methylation-specific primers. Images are representative of three experiments performed in duplicates.
these genes and/or markedly counteracted the stimulatory effect of TGFβ1 on most of them (figure 4D). Recent studies in lung fibroblasts demonstrated that besides glycolytic activation, TGFβ1 also triggers the expression of enzymes of the serine-glycine biosynthetic pathway, a key source of glycine critically needed for collagen synthesis.\(^{38-40}\) The serine-glycine biosynthetic pathway diverges from glycolysis via 3-phosphoglycerate, which in four consecutive steps is converted into glycine by the phosphoserine aminotransferase-1 (PSAT1), phosphoserine phosphatase (PSPH) and finally serine hydroxymethyltransferase-2 (SHMT2) (figure 4D).\(^{38-40}\) TGFβ1 induced the expression of the serine-glycine pathway genes, and this effect was blunted by CM272 (figure 4D), which also reduced the basal expression of PHGDH and the levels of H3K9 monomethylation by CM272 (figure 4D), which also reduced the basal expression of PHGDH and the levels of H3K9 monomethylation (figure 4D and online supplementary figure S7C), a transcriptional activating epigenetic modification mediated by G9a.\(^{41}\) Moreover, hypoxia-triggered expression of these genes in LX2 cells was also reduced by CM272 (online supplementary figure S8A). Noteworthy, the serine-glycine metabolic pathway is indeed important for the activation of LX2 cells, as indicated by the inhibitory effects of NCT503, a PHGDH enzymatic inhibitor,\(^{42}\) on hypoxia-elicited growth and TGFβ1-induced collagen synthesis in these cells (online supplementary figure S8B). Very interestingly, the expression of the rate-limiting gluconeogenic enzymes phosphoenolpyruvate-carboxykinase (PEPCK) and fructose-1,6-biphosphatase-1 (FBP1), repressed during fibrogenic activation,\(^{43}\) was also inhibited by TGFβ1, but restored under CM272 treatment (figure 4D). Moreover, the expression of the transcription factor and metabolic regulator peroxisome proliferator-activated receptor co-activator-1α (PGC-1α), recently identified as a key guardian of lung fibroblasts quiescence,\(^{39,47}\) was also repressed by TGFβ1 and was potently reactivated on CM272 treatment (figure 4D). These responses to CM272 were reproduced in human primary HSC (online supplementary figure S9A). Importantly, the upregulation of FBP1 and PGC-1α expression by CM272 (figure 4E) was related to the on-target pharmacological actions of this molecule. By qChIP analyses we found that CM272 reduced the levels of the repressive H3K9me2 mark in the proximal promoters of FBP1 and PGC-1α (figure 4F). At the DNA level, FBP1 promoter was found hypermethylated in a region previously associated with its transcriptional repression in cancer,\(^{26}\) and DNA methylation was reduced on CM272 or decitabine treatment (figure 4G). Regarding PGC-1α, we did not find significant levels of DNA methylation (figure 4G), suggesting that its transcriptional repression could be mainly mediated by G9a-H3K9 dimethylation, which indeed was reversed by CM272 treatment (figure 4F). In support of these notions, we observed that FBP1 expression was upregulated by decitabine or BIX01294, and together both agents had an additive effect, while PGC-1α expression was induced only by BIX01294 (online supplementary figure S9B).

CM272 inhibits hepatic fibrogenesis in vivo

Next, we examined the antifibrogenic potential of CM272 in different mouse models. First, we tested the effects of CM272 on the acute activation of HSC on single CCl\(_4\) injection. We found that CM272 administration 24 hours after CCl\(_4\) markedly inhibited HSC activation as indicated by α-SMA expression (online supplementary figure S10A). The antifibrogenic activity of CM272 was also evident in chronic liver injury. Mice received CCl\(_4\) twice a week for 6 weeks, and for the last 2 weeks were treated with CM272 or its vehicle (figure 5A). α-SMA and Sirius red staining for collagen deposition demonstrated reduced liver fibrosis in CM272-treated mice (figure 5A), corroborated by decreased expression of Coll1α1, α-Sma and Tgfb1 (figure 5B). Interestingly, expression of Pkm2, previously identified as a marker of glycolytic activation in liver myofibroblasts and a key regulator of glycolysis and the serine-glycine pathway,\(^{44,45}\) was induced by CCl\(_4\) administration. Noteworthy, Pkm2 expression was significantly attenuated by CM272 treatment, as was that of Phgdh (figure 5A and B). The antifibrotic effects of CM272 were reproduced in a model of cholestatic liver injury induced by BDL, as demonstrated by reduced α-SMA immunostaining, collagen deposition and expression of fibrogenesis-related and glycolysis-related genes (figure 5C,D). As in the CCl\(_4\) model, Pkm2 expression was also increased in areas of active fibrosis and was downregulated by CM272 (figure 5C). No significant differences in serum transaminases and creatinine levels nor body weight were found between vehicle and CM272-treated mice in either model, while a decrease in the hepatic expression of pro-inflammatory cytokines was noticed (online supplementary figure S10B,C). Together, these findings demonstrate that in vivo targeting of G9a/DNMT1 with CM272 during ongoing liver injury has antifibrotic potential and is exempt of overt toxicity.

CM272 has antifibrotic activity in human PCLSs

To further validate the antifibrotic effects of CM272, we used human PCLSs cultured in a newly designed bioreactor that allows modelling active fibrogenesis induced by pathophysiological stimuli; TGFβ1 and PDGF-BB.\(^{28}\) First, we observed that G9a, DNMT1 and UHRF1 expression was significantly increased after 96 hours in culture compared with freshly isolated tissues, and TGFβ1+PDGF-BB enhanced this response (figure 6A). Immunohistochemical analyses of PCLSs detected G9a and DNMT1 proteins in regions of the parenchyma enriched in α-SMA positive cells (figure 6B). Next, we tested the effects of CM272 treatment on TGFβ1+PDGF-BB-mediated fibrogenic activation of PCLSs (figure 6A). PCLSs were incubated with TGFβ1+PDGF-BB in the absence or presence of CM272 or the TGFβ1 receptor-1 inhibitor (Alk5i) SB-525334.\(^{28}\) As shown in figure 6C, the upregulation of fibrogenic gene expression elicited by TGFβ1+PDGF-BB was significantly attenuated by CM272. Consistently, soluble collagen secretion into the culture media, its deposition in the fibrotic matrix, and α-SMA staining were also markedly inhibited (figure 6D,E). Interestingly, lactate accumulation in the culture medium, indicative of metabolic glycolytic reprogramming, was inhibited by SB-525334 and also very efficiently by CM272 (figure 6F). Accordingly, FBP1 expression was downregulated by TGFβ1+PDGF-BB treatment while that of PKM2 and PHGDH was induced (figure 6G). These changes were also reversed by SB-525334 and CM272 (figure 6G). Immunohistochemical staining of PCLSs detected PKM2 expression in fibrogenic cells, validating the activation of glycolysis in human liver tissues by fibrogenic stimuli, and its inhibition by CM272 (figure 6H). Our PCLS model may also provide valuable information on potential hepatotoxic effects of experimental therapies in a human liver tissue environment.\(^{28}\) We measured a series of parameters, including albumin and urea levels, and lactate dehydrogenase (LDH), AST and ALT activities in conditioned media from control and CM272 treated PCLSs. We found no significant differences on these markers of hepatocellular function and injury in comparison with controls (figure 7A), and no major histological alterations on H&E staining were observed either (figure 7B).
Figure 5  CM272 inhibits liver fibrogenesis in vivo. (A) As shown in the diagram, mice received CCl₄ or vehicle (oil) for 6 weeks, and for the last 2 weeks were treated with CM272 (2.5 mg/kg body weight) or PBS. Animals were humanely killed 24 hours or 4 days after the last CCl₄ injection and liver tissues were immunostained for α-SMA and PKM2, or stained with Sirius red for collagen detection. Representative images are shown. (B) Expression of key genes involved in hepatic fibrogenesis and metabolic reprogramming in the livers of mice treated as described in (A). (C) Mice underwent bile duct ligation (BDL) and were treated with CM272 (2.5 mg/kg body weight) or PBS as indicated in the diagram. At day 11 after surgery, animals were sacrificed and liver tissue sections were immunostained for α-SMA and PKM2 or stained with Sirius red for collagen detection. (D) Expression of key genes involved in hepatic fibrogenesis and metabolic reprogramming in the livers of mice treated as described in the graph. Liver samples from sham-operated mice were used as controls.
Figure 6  CM272 has antifibrotic effects in human precision-cut liver slices (PCLSs). (A) Human PCLSs were isolated and placed in the bioreactor chambers. After 24 hours, PCLSs were treated with a fibrogenic stimulus (TGFβ1+PDGF-BB), its vehicle, CM272 (1 µM) or the activin receptor-like kinase 5 inhibitor (Alk5i) SB-525334 as shown in the graph. G9a, DNMT1 and UHRF1 expression levels were measured by qPCR. (B) Immunohistochemical analyses of G9a, DNMT1 and α-SMA performed in tissue sections from PCLSs treated as indicated. Representative images are shown. (C) qPCR analysis of the expression of key genes involved in hepatic fibrogenesis in PCLSs treated as indicated. (D) Soluble collagen (COLIA1) levels in media of bioreactor cultured PCLSs after 72 and 96 hours of incubation under the indicated conditions. Grey bars: vehicle; black bars: TGFβ1+PDGF-BB. (E) Representative images of α-SMA and picrosirius-red-stained tissue sections from PCLSs at t=0 and after 96 hours of treatment as indicated. (F) Quantification of lactate accumulation in media of bioreactor cultured PCLSs after 72 and 96 hours of incubation under the indicated conditions. Grey bars: vehicle; black bars: TGFβ1+PDGF-BB. (G) qPCR analysis of the expression of key genes associated with the reprogramming of glucose metabolism in PCLSs. (H) Immunohistochemical analysis of PKM2 performed in tissue sections from PCLSs at t=0 and after 96 hours of treatment as indicated. Representative images are shown. PCLSs from four different patients were used in four independent experiments. For each time point and condition, two PCLSs were used.
DISCUSSION

Accumulating evidence shows the involvement of epigenetic mechanisms in the activation of quiescent hepatic ECM-producing cells and the maintenance of their fibrogenic phenotype.9 11 Here, we confirmed the overexpression of DNMT1 in human and mouse fibrotic liver,44 and report the concomitant upregulation of the HMT G9a. Our novel findings indicate that these epigenetic effectors, together with their functional adaptor UHRF1,29 contribute to HSC fibrogenic activation. Besides their marked induction during primary mouse HSC activation in culture, we observed that TGFβ1 stimulation led to their fast recruitment to the chromatin-bound nuclear subfraction in LX2 cells. This response has been observed for other transcriptional regulators involved in TGFβ1 control of gene expression such as activating transcription factor-4.40 Here, we extend this dynamic effect of TGFβ1 to epigenetic factors. However, compelling evidence on the involvement of G9a, DNMT1 and UHRF1 in liver fibrogenic cell activation was obtained when their expression was inhibited (siRNAs) in LX2 cells, and we observed that the pro-fibrogenic transcriptomic response to TGFβ1 was abrogated.

This genetic evidence, together with the extensive functional crosstalk between different chromatin regulatory mechanisms, such as DNA and H3K9 methylation, prompted us to characterise in detail the antifibrogenic potential of a novel dual G9a/DNMT inhibitor CM272.24 We observed that CM272 markedly inhibited TGFβ1-stimulated pro-fibrogenic gene expression in LX2 and human primary HSC. Interestingly, these effects of CM272 were not restricted to TGFβ1 action, as they were also observed under hypoxia, another key proliferative and fibrogenic stimulus for HSC.32 33 To elucidate the mechanisms underlying CM272 activity, we performed transcriptomic studies in LX2 cells treated with TGFβ1 in the absence or presence of the drug. Consistent with the inhibition of TGFβ1-triggered fibrogenic activation, our GSEA found negative enrichment in categories associated with TGFβ1 and PDGFRB signalling pathways. Interestingly, the HIF pathway, which critically participates in TGFβ1-mediated kidney fibrogenesis,45 was also negatively affected by CM272. Notwithstanding the mechanistic relevance of these responses, it was the effect of CM272 on the expression of metabolism-related genes that captured our attention. Incipient but nonetheless robust evidence on the importance of metabolic reprogramming for fibrogenic cell activation is steadily accumulating. Similar to the Warburg effect in cancer cells, glycolytic activation along with mitochondrial dysfunction have been shown to contribute to fibrogenesis in different tissues.5 35–37 Early evidence obtained in liver myofibroblasts showed how reciprocal changes in glycolytic and gluconeogenic enzymes triggered by hedgehog signalling were mechanistically linked to HSC activation. Here, we found that TGFβ1 elicited very similar responses, inducing the expression of most genes coding for glycolytic enzymes and repressing that of the rate-limiting gluconeogenic genes FBP1 and PEPCK, as well as the metabolic regulator PGC-1α, which downregulation in lung fibroblasts markedly contributes to their activation.37 These transcriptional effects of TGFβ1 translated into metabolic alterations, including enhanced glycolytic rate and decreased mitochondrial activity. Consequently, ATP production shifted from a preferentially mitochondrial origin (OXPHOS) to a glycolytic one. In agreement with recent findings in lung myofibroblasts,38 we found that in human HSC TGFβ1 markedly stimulated the expression of genes in the serine–glycine pathway. This pathway is essential for the supply of glycine for collagen synthesis38 39 and also connects glycolysis with one-carbon metabolism and nucleotide synthesis, required for cell proliferation.42 We found that CM272 treatment effectively reversed the transcriptional programme triggered by TGFβ1 and its impact on glycolytic
activity and mitochondrial function. The molecular mechanisms underlying these effects are likely complex, but to a great extent may be attributed to specific pharmacological activities of CM272. We believe that one central target gene in the antifibrogenic effects of CM272 would be FBP1. As we showed, the expression of FBP1 is downregulated in activated liver myofibroblasts through epigenetic mechanisms involving increased DNA and H3K9 methylation in its promoter, modifications that were reversed by CM272. FBP1 is a key gluconeogenic enzyme and is also able to suppress HIF-1α activity by direct binding and acting as a transcriptional corepressor of HIF-1α target genes, which include most of glycolytic enzymes.45 Moreover, FBP1-mediated suppression of HIF-1α activity may also be involved in the antagonistic effects of CM272 on TGFβ1 responses, as the HIF-1α pathway is co-opted by TGFβ1 for its pro-fibrogenic activity even under normoxia.45,46 Regarding the normalization of mitochondrial function, together with FBP1 reactivation47 the enhanced expression of PGC-1α by CM272 treatment may also be relevant. PGC-1α is a master metabolic regulator, with roles including the preservation of mitochondrial function and the regulation of gluconeogenic gene expression (e.g., PEPCK).46 Recently, transcriptional repression of PGC-1α has been critically involved in lung myofibroblast metabolic reprogramming and activation.47 Interestingly, G9a-mediated H3K9 methylation was also shown to participate in PGC-1α repression during lung myofibroblast activation.15 Concomitantly, CM272 inhibition of G9a activity might also be involved in the repression of serine-glycine pathway genes, as G9a-mediated H3K9 monomethylation has been reported to mediate the transcriptional activation of these genes.41

Our in vitro observations were validated in two aetiologically distinct mouse models of liver fibrogenesis. Indeed, the expression of G9a and DNMT1 was detected in stromal fibrogenic cells also stained with α-SMA, and CM272 reduced myofibroblast activation and ECM accumulation. Mechanistically, the inhibitory effects of CM272 on fibrogenic metabolic reprogramming could also be taking place in vivo, as indicated by decreased accumulation of PKM2-expressing stromal cells. Importantly, these findings were extended to the human setting. Cirrhotic human liver tissues also showed increased levels of G9a and DNMT1 in areas of active fibrosis, and the expression of these epigenetic effectors was induced in cultured PCLSs concomitantly with their fibrogenic activation. PCLSs are a very useful tool to test antifibrotic drugs due to being a close surrogate of the human liver microenvironment.28 Here, we reproduced the antifibrogenic effects of CM272 observed in cultured cells and mouse models, including key aspects of HSC activation and metabolic reprogramming. One fundamental feature of any drug candidate is the absence of toxic reactions, particularly when intended to be administered to patients with liver injury. Consistent with our previous reports,28,29 we did not observe any signs of hepatic or systemic toxicity in mice treated with CM272. Most importantly, this lack of toxicity was also evident in human PCLSs, where parameters of hepatocellular function (eg, albumin production) and cell integrity were not negatively affected by the drug. Nevertheless, as some of us recently showed, there are emerging technologies allowing myofibroblast-selective drug delivery in vivo which may further enhance drug efficacy and safety in the context of liver injury.16

In summary, we have identified novel epigenetic targets involved in liver fibrosis and demonstrated that their dual targeting with an innovative ‘epi-drug’ can inhibit progression of liver fibrosis even in the absence of treating the underlying disease. We have also provided extended evidence on the role of metabolic reprogramming in liver fibrogenesis, and how this can be manipulated at the epigenetic level to halt or reverse the process. CM272 might be also considered for the treatment of fibrotic processes in other organs like the lung and kidneys, in which this condition has devastating effects.

**Author affiliations**

1Hepatology Program, CIMA, University of Navarra, IdiSNA, Pamplona, Spain
2Newcastle Fibrosis Research Group, Institute of Cellular Medicine, Newcastle University Faculty of Medical Sciences, Newcastle upon Tyne, UK
3Clinica Universidad de Navarra, CIBERehd, Pamplona, Spain
4Department of Biochemistry and Genetics, University of Navarra, Pamplona, Spain
5Department of Medical Biology, Pomar Medical University, Szczecin, Poland
6Department of General, Transplant and Liver Surgery, Warsaw Medical University, Szczecin, Poland
7Liver Unit. Department of Internal Medicine, Clinica Universidad de Navarra, IdiSNA, Pamplona, Spain
8North East’s Hepato-Pancreateo-Biliary (HPB) Centre, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle, UK
9Cell Therapy Program, Cima, University of Navarra, Pamplona, Spain
10Molecular Therapies Program, Cima, University of Navarra, Pamplona, Spain
11Oncohematology and Cell Therapy Programs, CIMA, University of Navarra, IdiSNA, Pamplona, Spain
12Institute for Liver and Digestive Health, Royal Free, University College London, UCL, London, UK
13CIBEREH, Madrid, Spain
14Hepatology Program, Centro de Investigación Medica Aplicada, Pamplona, Navarra, Spain

**Twitter** Maite G Fernandez-Barrena @Maite G Fernandez-Barrena

**Acknowledgements** We particularly acknowledge the patients for their participation and the Biobank of the University of Navarra for its collaboration. We thank Mr Roberto Barbero and Mrs Sara Arcelus for their technical support.

**Contributors** Performed experiments and data interpretation: MB-V, HP, LA, IU, MUL, ES, MR, MG, AC, LC, MA, MJL, MM, AP-S. Provided key biological samples and materials: PM, BS, SMR, JE, FO, PF, KR, FO, JM. Critical revision of the manuscript: JO, PP, KR, FO, JM, MIJ, CB. Study design, data interpretation, manuscript writing and submission: CB, MGB-F, MAA.

**Funding** We thank the financial support of CIBERehd; grant P16/01126 from Instituto de Salud Carlos III (ISCIII) co-financed by “Fondo Europeo de Desarrollo Regional” (FEDER) “Una manera de hacer Europa”; grant 58/17 from Gobierno de Navarra; grants SAF2014-54191-R, SAF2017-88933-R and SAF2019-104878RB-100 from FEDER/Ministerio de Ciencia, Innovación y Universidades-Agenzia Estatal de Investigación; grant BIO15/CA/011 from Bio-Euskadi Fundazioa (Eibb maratobia); grant from Asociación Española Contra el Cáncer (AEC) Scientific Foundation Rare Cancers grant 2017; HEPACARE Project from Fundación La Caixa; Fundación Eugenio Rodríguez Pascual; Fundación Echávez; Fundación Mario Losantos and Fundación M Torres. We thank Mr Eduardo Ávila and Mr Sergio Durá for their generous contribution. FPI fellowships from Ministerio de Educación, Cultura y Deporte to MB-V; MG and MR; FIMA-CIMA fellowship to AC; Gobierno de Navarra fellowship to LC; AECC post-doctoral fellowship to MA and Ramón y Cajal Program contract to MGB-F. This work was also funded by a UK Medical Research Council programme grants to JM, FO and others (MR/K01019494/1, MK/K001949/1, MR/R023026/1); National Institute on Alcohol Abuse and Alcoholism (NIAAA) (grant U01AA018663). The research was also supported by the National Institute for Health Research Newcastle Biomedical Research Centre based at Newcastle Hospitals NHS Foundation Trust and Newcastle University.

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not required.

**Ethics approval** This study was approved by the Newcastle & North Tyneside Research Ethics Committee. Human samples were processed following standard operating procedures approved by the Ethical and Scientific Committees of the University of Navarra and the Medical University of Warsaw. Animal care and procedures were approved by the Animal Care Committee of the University of Navarra or the Newcastle Animal Welfare and Ethical Review Board and performed under a UK Home Office license.

**Provenance and peer review** Not commissioned; externally peer reviewed.
Data availability statement Data are available in a public, open access repository. GEO repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139504).

ORCID iDs
Marina Barcena-Varela http://orcid.org/0000-0001-7868-7939
Maria Arechedera http://orcid.org/0000-0002-4830-1924
Carmen Berasain http://orcid.org/0000-0001-7075-2476
Matias A Avila http://orcid.org/0000-0001-6570-3557
Maite G Fernandez-Barrena http://orcid.org/0000-0003-4459-5301

REFERENCES
1 Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol 2011;6:425–56.
2 Hernandez-Gea V, Toffanin S, Friedman SL, et al. Role of the microenvironment in the pathogenesis and treatment of hepatic fibrosis. Gastroenterology 2013;144:512–27.
3 Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol 2017;14:397–411.
4 Chen Y, Choi SS, Michelotti GA, et al. CBX5/G9a/H3K9me- mediated gene repression is essential to fibroblast activation during lung fibrosis. JCI Insight 2016;89:147–57.
5 Hou W, Syn W-K. Role of metabolism in hepatic stellate cell activation during liver fibrosis and carcinogenesis. Int J Mol Sci 2015;20:2507.
6 Troeger JS, Mederacke I, Gwak G-Y, et al. Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. Gastroenterology 2012;143:e11:1319–29.
7 Kisseleva T, Cong M, Paik Y, et al. Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc Natl Acad Sci U S A 2012;109:9484–53.
8 Lee VA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut 2015;64:830–41.
9 Morán-Salvador E, Mann J. Epigenetics and liver fibrosis. Cell Mol Gastroenterol Hepatol 2017;4:125–34.
10 Barcena-Varela M, Colvin L, Fernandez-Barrena MG. Epigenetic mechanisms in hepatic stellate cell activation during liver fibrosis and carcinogenesis. Int J Mol Sci 2019;20:2507.
11 Dong C, Yuan T, Wu Y, et al. Loss of fbp1 by Snail- mediated repression provides metabolic advantages in basal- like breast cancer. J Med Chem 2018;61:6518–45.
12 Bárz-E, Zelál J, Gondorovics K, et al. Functional role of G9a histone methyltransferase in cancer. Front Immunol 2015;6:487.
13 San José-Enríquez E, Aguirre X, Rabal O, et al. Discovery of first-in-class reversible dual small molecule inhibitors against G9a and DNMTs in hematological malignancies. Nat Commun 2017;8:15424.
14 Ye J, Mancuso A, Tong X, et al. Discovery of reversible DNA methyltransferase and histone methyltransferase G9a inhibitors with antitumoral in vivo efficacy. J Med Chem 2018;61:6518–45.
15 García-Lloigny Q, Carroti S, Latasa MU, et al. Matrix metalloproteinsae-10 expression is induced during hepatic injury and plays a fundamental role in liver tissue repair. Liver Int 2014;34:e257–70.
16 Bárz-E, Zelál J, Gondorovics K, et al. Fate-mapping evidence that hepatic stellate cells are epithelial progenitors in adult mouse livers. Stem Cells 2008;26:2104–13.
17 Roth KJ, Copple BL. Role of hypoxia-inducible factors in the development of liver fibrosis. Cell Mol Gastroenterol Hepatol 2015;1:589–97.
18 Wang M, Wang Q, Li Y, et al. Inhibition of hypglycolysis in mesothelial cells prevents peritoneal fibrosis. Sci Transl Med 2019;11:eava5341.
19 Caporrelli N, Meranaka RA, Melton AY, et al. Transforming growth factor (TGFB)-promotes de novo serine synthesis for collagen production. J Biol Chem 2016;291:27239–51.
20 Xie N, Tan Z, Banerjee S, et al. Glycolytic reprogramming in myofibroblast differentiation and lung fibrosis. Am J Respir Crit Care Med 2019;121462–74.
21 Li B, Qiu B, Lee DSM, et al. A histone deacetylase inhibitor, trichostatin attenuates renal fibrosis and retains Klotho expression. Gastroenterology 2016;150.
22 Niki T, Rombouts K, De Bleser P, et al. Deactivation of hepatic stellate cells during liver fibrosis progression. Nat Chem Biol 2012;8:1073–83.
23 Garcia-Lloigny Q, Carroti S, Latasa MU, et al. Metallixxproteinase-10 expression is induced during hepatic injury and plays a fundamental role in liver tissue repair. Liver Int 2014;34:e257–70.
24 Paish HL, Reed LH, Brown H, et al. A bioreactor technology for modeling fibrosis in human and rodent precision-cut liver slices. Hepatology 2019;70;hep30651:1377–91.
25 Ferrer L, Fournier A, Tsuasa T, et al. Methylation of DNA ligase 1 by G9a/GLP recruits UHRF1 to replicating DNA and regulates DNA methylolation. Mol Cell 2017;64:455–65.
26 Li B, Qiu B, Lee DSM, et al. Matrix metalloproteinsae-10 expression is induced during hepatic injury and plays a fundamental role in liver tissue repair. Liver Int 2014;34:e257–70.
27 García-Lloigny Q, Carroti S, Latasa MU, et al. Matrix metalloproteinsae-10 expression is induced during hepatic injury and plays a fundamental role in liver tissue repair. Liver Int 2014;34:e257–70.