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The hepatocyte epidermal growth factor receptor (EGFR) pathway regulates the cellular interactome within the liver fibrotic niche

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Abstract

Liver fibrosis is the consequence of chronic liver injury in the presence of an inflammatory component. Although the main executors of this activation are known, the mechanisms that lead to the inflammatory process that mediates the production of pro-fibrotic factors are not well characterized. Epidermal growth factor receptor (EGFR) signaling in hepatocytes is essential for the regenerative processes of the liver; however, its potential role in regulating the fibrotic niche is not yet clear. Our group generated a mouse model that expresses an inactive truncated form of the EGFR specifically in hepatocytes (Δ EGFR mice). Here, we have analyzed the response of WT and Δ EGFR mice to chronic treatment with carbon tetrachloride (CCl₄), which induces a pro-inflammatory and fibrotic process in the liver. The results indicated that the hallmarks of liver fibrosis were attenuated in CCl₄-treated Δ EGFR mice when compared with CCl₄-treated WT mice, coinciding with a faster resolution of the fibrotic process and ameliorated damage. The absence of EGFR activity in hepatocytes induced changes in the pattern of immune cells in the liver, with a notable increase in the population of M2 macrophages, more related to fibrosis resolution, as well as in the population of lymphocytes related to eradication of the damage. Transcriptome analysis of hepatocytes, and secretome studies of extracellular media from *in vitro* experiments, allowed us to elucidate the specific molecular mechanisms regulated by EGFR that mediate hepatocyte production of both pro-fibrotic and pro-inflammatory mediators; these have consequences for the deposition of extracellular matrix proteins, as well as for the immune microenvironment. Overall, our study uncovered novel mechanistic insights regarding EGFR kinase-dependent actions in hepatocytes that reveal its key role in chronic liver damage.

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Keywords: intercellular crosstalk; secretome; inflammatory response; cytokines; extracellular matrix; liver fibrosis; CCl₄; macrophages; immune microenvironment; liver damage

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No conflicts of interest were declared.

Introduction

Fibrosis is a highly conserved and coordinated, initially protective, response to tissue injury. The interaction among multiple pathways determines whether fibrosis is self-limiting and homeostatic or whether it is uncontrolled and excessive [1]. When a chronic injury takes place in the liver, mobilization of lymphocytes and other inflammatory cells occurs, thus setting the stage for persistence of an inflammatory response. Macrophages produce pro-fibrotic mediators that are responsible for the activation of quiescent hepatic stellate cells (HSCs) to a myofibroblast (MFB) phenotype. MFBs are the principal source of extracellular matrix (ECM) protein accumulation and prominent mediators of fibrogenesis [2].

The epidermal growth factor receptor encoded by the gene *EGFR* (previously known as *ERBB1*) is a classical tyrosine kinase receptor that belongs to the family of ErbB receptors, and it is highly expressed in the liver. The EGFR pathway regulates hepatocyte proliferation, playing important roles in liver regeneration and hepatocellular carcinogenesis [3]. But the EGFR signaling system has also been identified as a key player in all stages of the liver's response to injury, from early inflammation and hepatocellular proliferation to fibrogenesis and neoplastic transformation [4]. The role of EGFR in chronic liver injury and fibrosis is also relevant for human pathophysiology, because EGF, EGFR, and phospho-EGFR levels increase in the liver of cirrhotic or metabolic dysfunction-associated steatotic liver (MAFLD) patients [3]. A direct role of EGFR, or its ligands, in HSC activation and subsequent ECM modification has been suggested, concomitant with regulation of steatosis, in different *in vitro* and *in vivo* animal models of liver fibrosis and MAFLD [5–9]. However, different studies have pointed out that the EGFR signaling might be protective in other processes of liver injury and fibrosis [10,11].

With the aim of better understanding the specific role of the hepatocyte EGFR pathway in liver physiology and pathology, some years ago we generated a transgenic mouse model expressing a truncated form of human EGFR specifically in hepatocytes, which acts as dominant negative mutant (Δ EGFR) and allows definition of its tyrosine kinase-dependent functions [12]. We found that EGFR catalytic activity is critical in the early preneoplastic stages of the liver because Δ EGFR mice showed a delay in the appearance of diethyl-nitrosamine (DEN)-induced tumors, which correlated with a strong attenuation and delay in the DEN-induced inflammatory process [12]. We also found that after chronic feeding with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), which induced cholestatic damage, Δ EGFR mice presented with a more efficient regeneration, concomitant with a shift from a pro-fibrotic to a restorative inflammatory response [13]. Previous studies had suggested that pro-inflammatory stimuli could mediate transactivation of the EGFR pathway, which would contribute to carcinogenesis

in inflammatory liver diseases [14,15]. However, our data pointed to a role for the EGFR pathway in hepatocytes, upstream of the inflammatory process. In this context, further investigations were required to comprehensively study the direct role of EGFR in hepatocytes in regulating inflammation during chronic liver injury.

Accordingly, the aim of this study was to elucidate whether the catalytic activity of the EGFR in hepatocytes could participate in regulating the inflammatory/pro-fibrotic environment in response to a chronic liver insult.

Materials and methods

Ethics approval statement

Animal protocols were approved by the Animal Experimentation Ethics Committee of the UCM and the Animal Welfare Division of the Environmental Affairs Council of the Government of Madrid (Proex 262.6/21).

In vivo experimental procedures

The novel transgenic mouse model that expresses a hepatocyte-specific truncated form of human EGFR (supplementary material, Figure S1A), which acts as dominant negative mutant (Δ EGFR) and allows definition of its tyrosine kinase-dependent functions, was used [12]. Chronic liver injury was induced by treatment with carbon tetrachloride (CCl_4 ; Sigma-Aldrich, St Louis, MO, USA). For this, 0.48 g/kg body weight CCl_4 , 1:10 v/v in mineral oil (MO), or vehicle (MO), was administered to 8-week-old C57BL/6 male mice by intraperitoneal injection (3 μ l/g body weight) twice a week for 4 or 8 weeks. At these timepoints, animals were sacrificed, and blood and liver tissue were collected (supplementary material, Figure S1B). The transgenic Δ EGFR mouse line is maintained in heterozygosity; therefore wild-type (WT) and Δ EGFR mice belong to the same strain. The WT and Δ EGFR mice used for experiments belonged to the same litters. We proved that Δ EGFR mice continued expressing the truncated human transgene following the CCl_4 treatment (supplementary material, Figure S1C). To confirm that CCl_4 was similarly metabolized in both groups, we analyzed the expression of *Cyp2b10*, whose expression increased similarly after the treatment in WT and Δ EGFR livers (supplementary material, Figure S1D). Moreover, since CCl_4 is a Cyp2e1 suicide substrate whose binding leads to its inactivation and degradation [16], we analyzed its expression, observing a similar decrease in both cases. These results suggest that CCl_4 was equally metabolized in all experimental groups (supplementary material, Figure S1D).

Detailed information about materials and methods and statistical analysis may be found in Supplementary materials and methods.

Results

Inactivation of EGFR in hepatocytes ameliorates CCl₄-induced liver damage and fibrosis

We first examined the consequences of inactivating EGFR signaling in hepatocytes on the liver damage progression after 4 or 8 weeks of CCl₄ chronic treatment. As observed in Figure 1A, serum levels of transaminases increased after 8 weeks of treatment in WT mice (no differences were observed at 4 weeks of treatment; results not shown). However, no significant increase was observed in ΔEGFR mice. Histological analysis also revealed less damage in the liver parenchyma after 8 weeks of treatment (Figure 1B), correlating with decreased levels of alpha-smooth muscle actin (α-SMA), characteristic of activated MFB (Figure 1C,D). In ΔEGFR livers, the expression and deposits of α-SMA increased after 4 weeks but decreased at 8 weeks, reflecting resolution of fibrosis. Specific analysis of the process of fibrosis revealed that the increase in the deposits of collagen fibers, caused by the CCl₄ treatment, was significantly lower in ΔEGFR livers (Figure 2A). The expression of different collagen genes, such as *Coll1a1* or *Col3a1*, increased significantly after 4 weeks of treatment; however, increases in expression were lower in ΔEGFR livers, which was particularly relevant for *Col3a1* (Figure 2B). It was very interesting to find differences in lysyl oxidase-related genes (responsible for the inter-chain polypeptide cross-linking of collagens), which were significantly increased in WT but not in ΔEGFR livers after 4 weeks of CCl₄ treatment (Figure 2B). ΔEGFR mice presented different patterns in the expression of metalloprotease mRNAs, particularly *Mmp2* and *Mmp9*, with a potential role in the resolution of fibrosis, which were significantly increased in ΔEGFR, but not in WT livers, after 8 weeks of treatment (Figure 2C). The expression of *Timp1* (TIMP metalloprotease inhibitor 1) was significantly lower in ΔEGFR livers after 4 weeks of CCl₄ treatment (Figure 2C), which would indicate a higher metalloprotease activity. Expression of other members of the family, such as *Timp3*, did not change.

We could not find relevant differences among WT and ΔEGFR livers in the expression of genes related to the transforming growth factor-beta (TGF-β) pathway, as one of the major pro-fibrotic factors. Thus, although the increase in some of its ligands appeared to be delayed in ΔEGFR mice (supplementary material, Figure S2A), phosphorylation of Smad3, a hallmark of the activation of the pathway, was significantly increased after 8 weeks of treatment in both WT and ΔEGFR livers (supplementary material, Figure S2B). No clear differences were observed in the expression of other pro-fibrotic genes, such as *Ccn2* (CTGF) or *Pdgfb* (supplementary material, Figure S3).

ΔEGFR livers showed significant differences in macrophage populations in the CCl₄ model of liver injury

With the aim of comparing the inflammatory response in WT and ΔEGFR mice treated with CCl₄, we first

analyzed the expression of *Adgre1* at the mRNA and protein levels (immunohistochemistry of F4/80 in tissue sections), as well as the expression of general inflammatory cytokines, such as *Tnf* or *Il1b* (supplementary material, Figure S4). No significant differences could be observed. Next, we isolated and analyzed by flow cytometry the intrahepatic immune monocyte/macrophage populations after 4 weeks of treatment. No increase in the percentage of resident macrophages was observed (Figure 3A). However, ΔEGFR livers presented a decrease in the late recruited macrophages (correlating with the less parenchymal damage observed in Figure 1) and the most significant result was the shift in the M1/M2 balance towards M2 polarity (Figure 3C), which correlated with a significantly lower ratio between the expression of the pro-inflammatory cytokine *Il12b*, characteristic of M1 macrophages, and the anti-inflammatory cytokine *Il10*, characteristic of M2 polarization, detected in ΔEGFR livers (Figure 3D). Regarding lymphocyte populations, a shift in the Cd4/Cd8 ratio was observed in favor of Cd4 lymphocytes in ΔEGFR livers (supplementary material, Figure S5), concurring with significantly lower levels of naïve and IL17⁺ Cd4⁺ lymphocytes, and a relevant increase in the Treg cell subpopulation (supplementary material, Figure S5).

The catalytic activity of EGFR regulates the hepatocyte gene transcriptome in response to CCl₄-induced liver damage

To deepen our understanding of underlying molecular mechanisms by which the EGFR pathway in hepatocytes could be controlling the fibrotic niche interactome, we next performed RNA sequencing (RNA-seq) analysis in hepatocytes isolated from WT and ΔEGFR mice treated with either MO or CCl₄ for 4 weeks (supplementary material, Figure S6A). The most relevant results are summarized in Figure 4. When the changes in gene expression caused by CCl₄ treatment versus control samples in both WT and ΔEGFR hepatocytes were compared, 1,258 genes were specifically differentially expressed in the WT comparison, whereas only 209 genes were differentially expressed in the one from ΔEGFR (Figure 4A). Transcription factor (TF) activity was inspected by functional enrichment analysis, based on the differentially expressed genes of each comparison. Treated hepatocytes from WT mice showed 265 TF binding sites significantly enriched among those genes, which did not appear in ΔEGFR mice (Figure 4B and see details in supplementary material, Figure S6B). Many of the TFs whose activity appeared modulated in WT hepatocytes were related to proliferation and differentiation (EGR, Myc, E2F, Ets-related, KLFs, etc.), stress pathways (HIF-1, p53), and metabolism (PPARα, SREBP, CREB-related). The most significant difference in the ΔEGFR hepatocytes was the increase in the activity of PPARγ, as well as GLI or NF1. In addition to the expected differences in the activation of the EGFR pathway (supplementary material, Figure S7A), relevant differences in tissue remodeling, collagen metabolic processes, or regulation

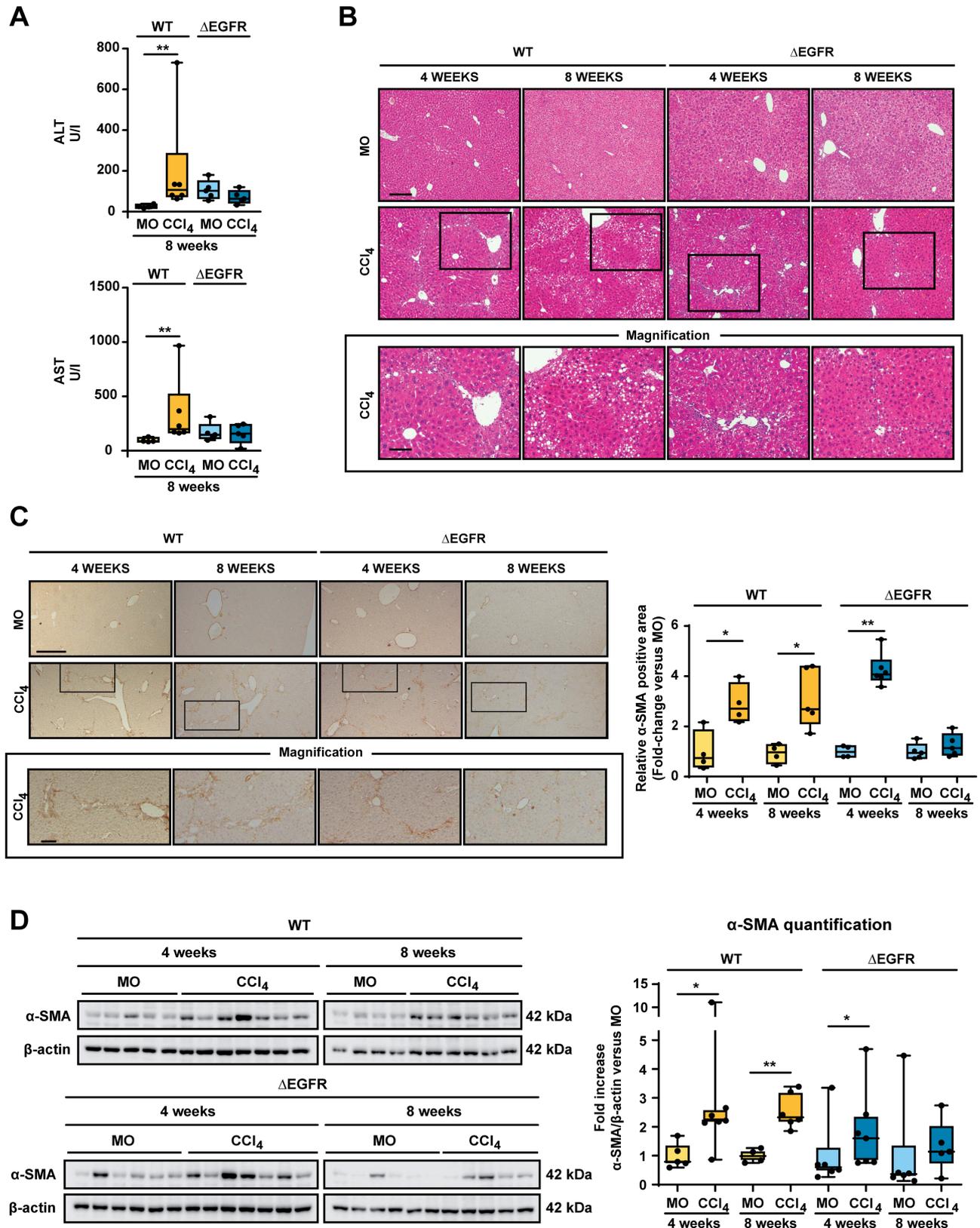


Figure 1. The impairment of EGFR catalytic activity in hepatocytes attenuates CCl₄-induced liver damage and myfibroblast activation. (A and B) Serum concentrations of alanine- and aspartate-aminotransferases (ALT and AST, respectively) (A) and hematoxylin and eosin-stained liver sections (B) were analyzed to assess CCl₄-induced liver damage. (C and D) Alpha-smooth muscle actin (α-SMA) expression was evaluated by immunostaining (quantification on the right) (C) and western blotting (densitometric analysis respect to β-actin on the right) (D), and data are expressed as fold-change versus mineral oil (MO). *n* = 4–7 animals per group. **p* < 0.05, ***p* < 0.01 using Student's *t*-test. Scale bars: 500 and 100 μm (magnified views).

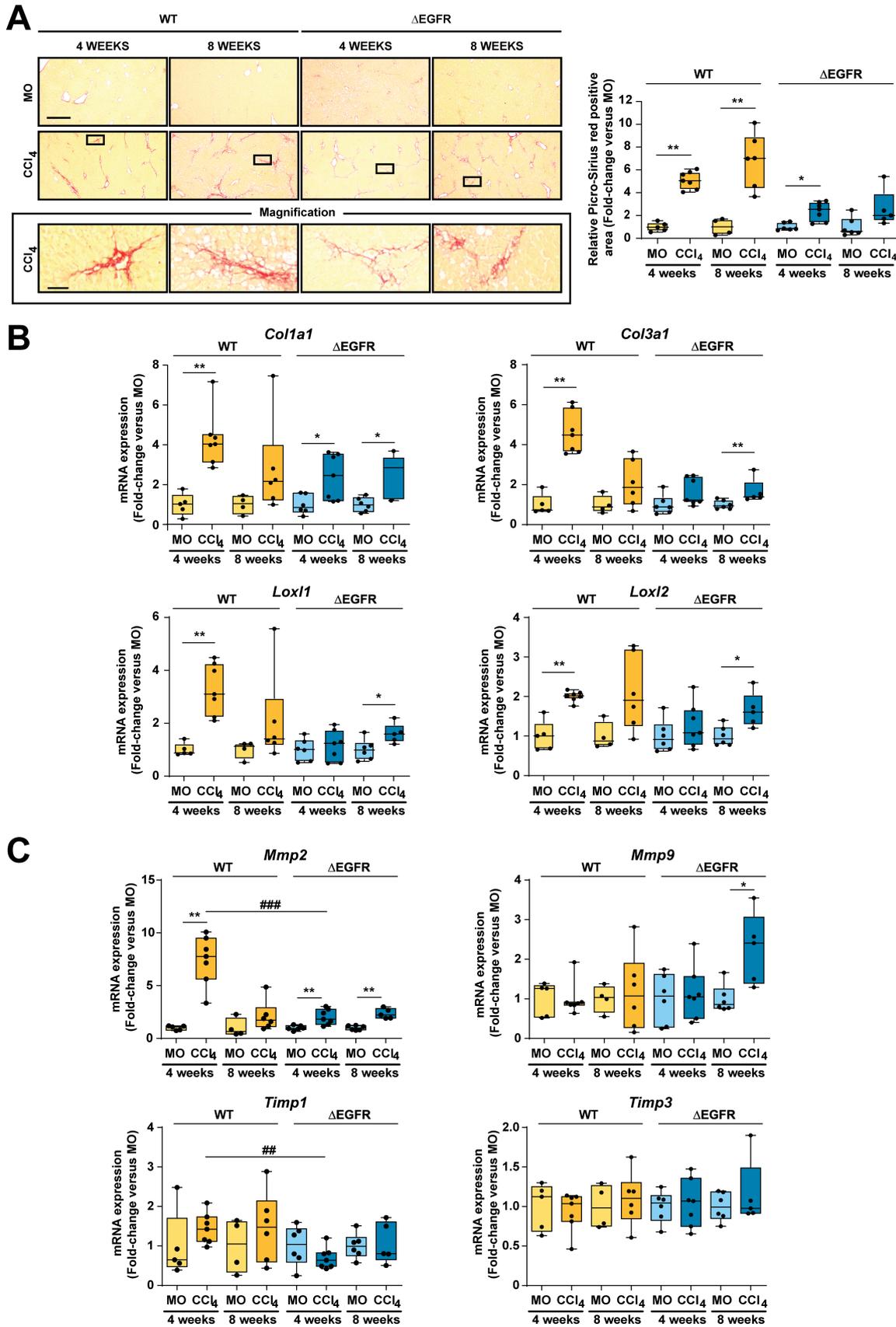


Figure 2. The impairment of EGFR catalytic activity in hepatocytes attenuates CCl₄-induced liver fibrosis. (A) Collagen deposition was assessed using picro-sirius red staining and the positive-stained area was quantified as shown on the right. (B and C) RT-qPCR analysis of the hepatic mRNA expression of genes involved in extracellular matrix remodeling: *Col1a1*, *Col3a1*, *Lox1*, *Lox2*, *Mmp2*, *Mmp9*, *Timp1*, and *Timp3*. Data ($n = 4-7$ animals per group) are expressed as fold-change versus mineral oil (MO). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, using Student's t -test. *CCl₄ versus MO; # ΔEGFR versus WT. Scale bars: 500 and 100 μm (magnified views).

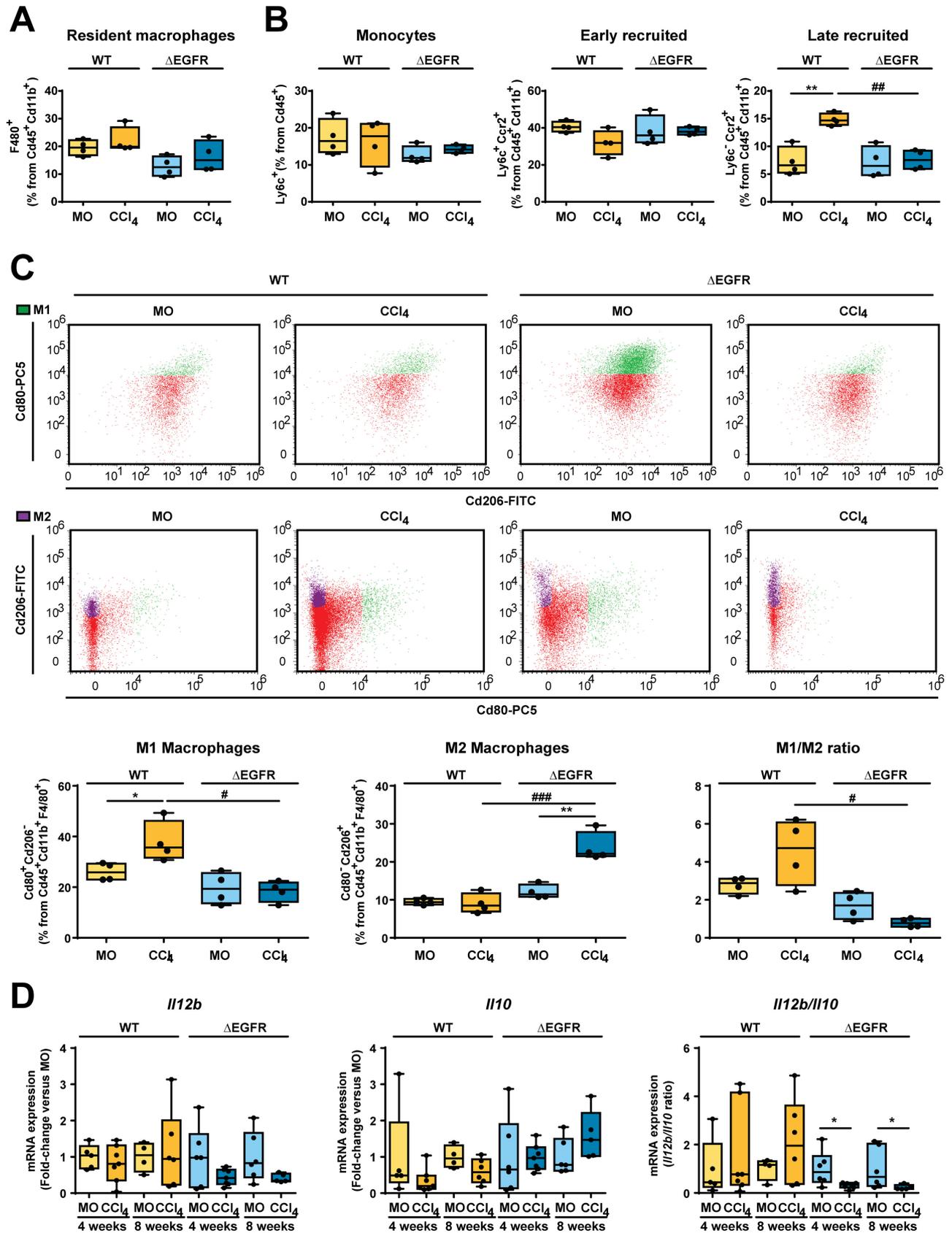


Figure 3. The impairment of EGFR catalytic activity in hepatocytes alters the macrophage phenotype following CCl₄-induced liver damage. (A–C) Non-parenchymal liver cells from WT and ΔEGFR mice after 4 weeks of CCl₄ treatment ($n = 4$ animals per group) were analyzed by flow cytometry: (A) resident macrophages; (B) monocytes and early- and late-recruited macrophages; (C) M1 and M2 macrophage populations and M1/M2 ratio. (D) Hepatic mRNA expression of *Il12b* (M2) and *Il10* (M1) analyzed by RT-qPCR (fold-change versus mineral oil, MO) and *Il12b/Il10* ratio. $n = 4$ –7 animals per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, using Student's *t*-test. *CCl₄ versus MO; #ΔEGFR versus WT.

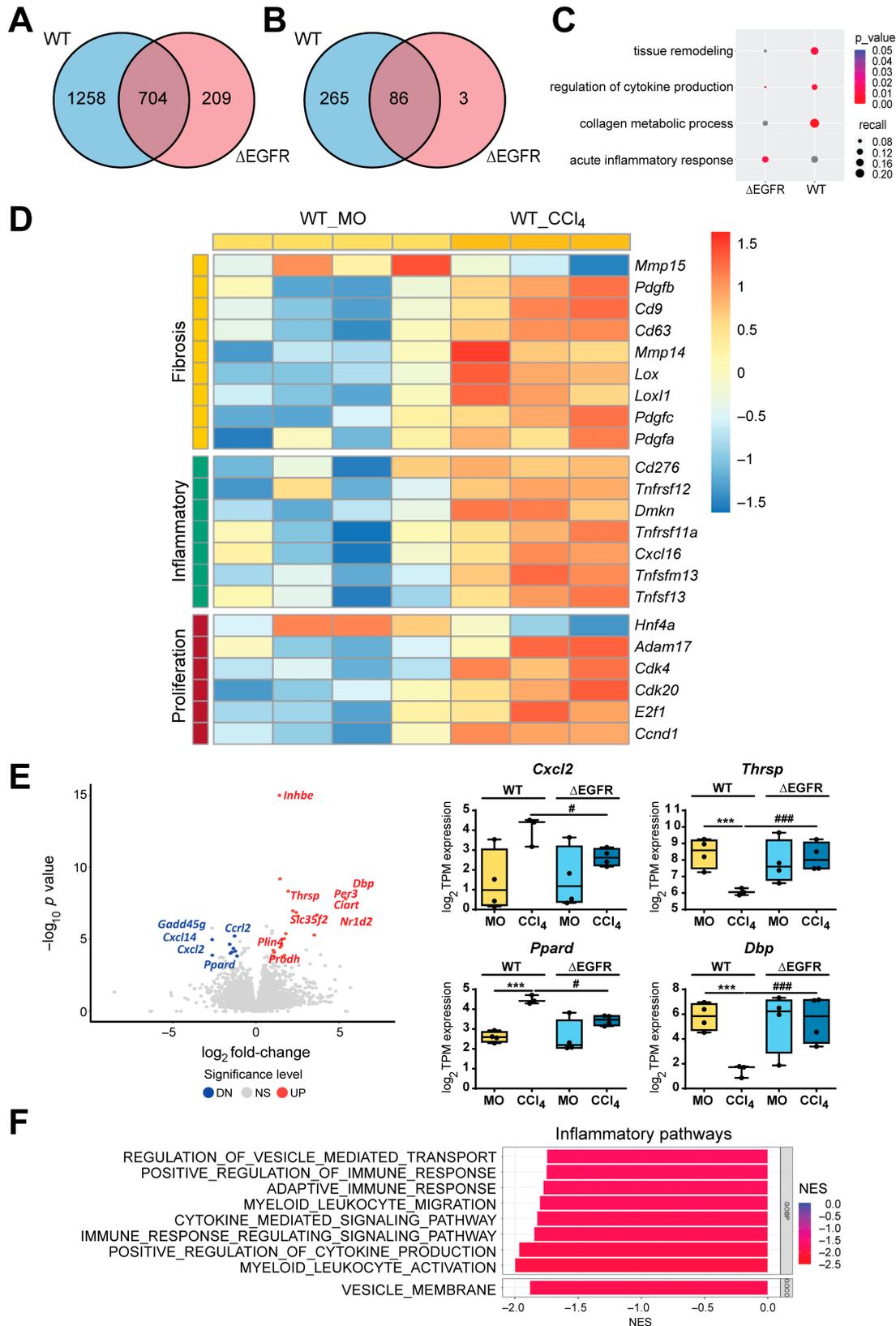


Figure 4. The EGFR pathway regulates the hepatocyte gene transcriptome in response to CCl₄. RNA-seq analysis in hepatocytes from WT and ΔEGFR mice treated with CCl₄ or mineral oil (MO) for 4 weeks (3 or 4 animals per group). (A and B) Venn diagrams showing (A) differentially expressed genes and (B) enriched transcription factor binding sites when comparing CCl₄-treated versus MO-treated mice (WT or ΔEGFR). (C) Dotplot showing enrichment differences for key events in chronic liver damage. (D) Heatmap showing changes in fibrosis-, inflammation-, and proliferation-related gene expression exclusively observed in CCl₄-treated WT mice. (E) Left: volcano plot showing differences in the expression of genes in ΔEGFR versus WT hepatocytes after CCl₄ treatment (red: upregulated; blue: downregulated; grey: non-significant). Right: mRNA expression (RT-qPCR) of some genes selected from the volcano plot. (F) Bar chart of GSEA-enriched inflammatory pathways after CCl₄ treatment (ΔEGFR versus WT). **p* < 0.05, ***p* < 0.01, ****p* < 0.001. *CCl₄ versus MO; # ΔEGFR versus WT.

of inflammation and cytokine production pathways were identified in hepatocytes from WT but not from Δ EGFR mice (Figure 4C). The cellular response to xenobiotic stimulus was activated in WT and Δ EGFR mice (supplementary material, Figure S7A), which corroborates that the response to CCl₄ has not been affected by silencing EGFR in hepatocytes.

Among the genes specifically regulated in hepatocytes from CCl₄-treated WT mice that did not appear modulated in Δ EGFR hepatocytes, we found genes related to fibrogenesis, such as members of the *Pdgf* family, metalloproteases (*Mmp15* or *Mmp14*), and lysyl oxidases or lysyl oxidase-like genes (*Lox* and *Lox11*), among others (Figure 4D), as well as genes involved in regulation of inflammation, such as cytokines/chemokines or genes related to TNF receptor signaling (Figure 4D). A volcano plot showing differences in the expression of genes when comparing Δ EGFR versus WT hepatocytes isolated after 4 weeks of CCl₄ treatment revealed a significant decrease in the expression of inflammatory genes, such as the cytokines *Cxcl2* or *Cxcl14* or the chemokine receptor *Ccr12* (Figure 4E, left). In fact, Gene Ontology (GO) enrichment analysis also revealed that Δ EGFR cells presented a significant decrease in inflammatory pathways (Figure 4F). The volcano representation also revealed differences in genes related to metabolism, such as *Ppard*, *Dbp*, or *Thrsp* (Figure 4E, left). In most of the cases, CCl₄ treatment induced changes in gene expression in WT hepatocytes, which were not observed in Δ EGFR hepatocytes (Figure 4E, right).

It is also worth mentioning that hepatocytes from CCl₄-treated WT mice, but not those from Δ EGFR mice, showed relevant changes in the expression of genes related to cell cycle progression and cell proliferation (Figure 4D), which would indicate higher hepatocyte regeneration in the WT than in the Δ EGFR mice. In parallel, hepatocyte identity genes, such as *Hnf4a* (Figure 4D), or genes related to liver-specific functions (supplementary material, Figure S7B), were downregulated. Interestingly, the Δ EGFR hepatocytes from CCl₄-treated mice showed higher expression of mitochondrial metabolism-related pathways, such as fatty acid catabolic processes, mitochondrial respiratory chain, aerobic electron transport chain, or ATP synthesis than those isolated from the WT mice (supplementary material, Figure S7C), which indicated that they are metabolically more efficient. Relative upregulation of TGF- β pathway-related genes, as well as epithelial–mesenchymal transition (EMT) gene signature (as defined in supplementary material, Table S5), was observed in hepatocytes after CCl₄ treatment, but changes were similar in WT and Δ EGFR hepatocytes (supplementary material, Figures S8 and S9).

Overall, our results indicate that in response to a chronic insult, the EGFR pathway in hepatocytes modulates some of the transcriptional program changes.

The catalytic activity of EGFR in hepatocytes regulates the inflammatory and pro-fibrotic secretome in response to CCl₄-induced liver damage. We previously generated immortalized hepatocytes from WT and Δ EGFR mice [12], which are a useful tool

for *in vitro* approaches. Here, we have analyzed how the secretome of these cells may affect the fibrotic cell interactome in *in vitro* experiments, focusing on the effects on macrophages. We incubated the WT and Δ EGFR hepatocytes with heparin-binding EGF-like growth factor (HB-EGF), a ligand of the EGFR, to activate the pathway (Figure 5A,B). We next followed the experimental set-up depicted in Figure 5C to generate conditioned medium that was used to analyze the effects on the phenotype of a mouse macrophage cell line (RAW264.7 cells). As observed in Figure 5D, the conditioned medium of WT and Δ EGFR hepatocytes produced opposite effects on the expression profile of genes associated with an M1 phenotype (pro-inflammatory/fibrotic), such as *Il12b*, or M2 (resolution of fibrosis), such as *Il10*. The ratio *Il12b/Il10* significantly increased only in RAW264.7 cells incubated with the conditioned medium from HB-EGF-treated WT hepatocytes.

To analyze whether the EGFR pathway would also be regulating the human macrophage phenotype, we used a human liver tumor cell line, Hep3B, and a human monocyte cell line, THP-1. Using a similar experimental approach (supplementary material, Figure S10A), conditioned medium of Hep3B cells untreated or treated with HB-EGF was collected to analyze the effects on THP-1 cells. The results indicated that the conditioned medium from HB-EGF-treated Hep3B cells increased monocyte adhesion (supplementary material, Figure S10B) and the expression of pro-inflammatory genes (M1 phenotype), downregulating the expression of anti-inflammatory-related genes (M2 phenotype) (supplementary material, Figure S10C). The *Il12b/Il10* ratio significantly increased in THP-1 cells after the incubation with the conditioned medium from HB-EGF-treated Hep3B cells (supplementary material, Figure S10D).

Overall, our results indicate that hepatocytes may actively participate by secreting factors that regulate the inflammatory microenvironment in the fibrotic niche. To validate this, we next performed a proteomic analysis of conditioned medium from hepatocytes obtained following the same experimental approach presented in Figure 5C. The most significant results are presented in Figure 5E. WT hepatocytes responded to HB-EGF by inducing the secretion of *Cxcl10*, *Cxcl12*, and *Cxcl16*, and this increase was not observed for Δ EGFR hepatocytes, reinforcing the role of hepatocytes in the secretion of inflammatory cytokines under the control of the EGFR pathway. Moreover, it was also very interesting to observe that WT hepatocytes responded to HB-EGF by inducing the secretion of different proteins involved in fibrosis, among them, different members of collagens, or proteins involved in their maturation, such as *Lox11*. Δ EGFR hepatocytes secreted much lower levels of all these proteins, and as expected, no changes were observed in response to HB-EGF. Indeed, the secretome analysis also revealed a role for hepatocytes in the production of ECM proteins.

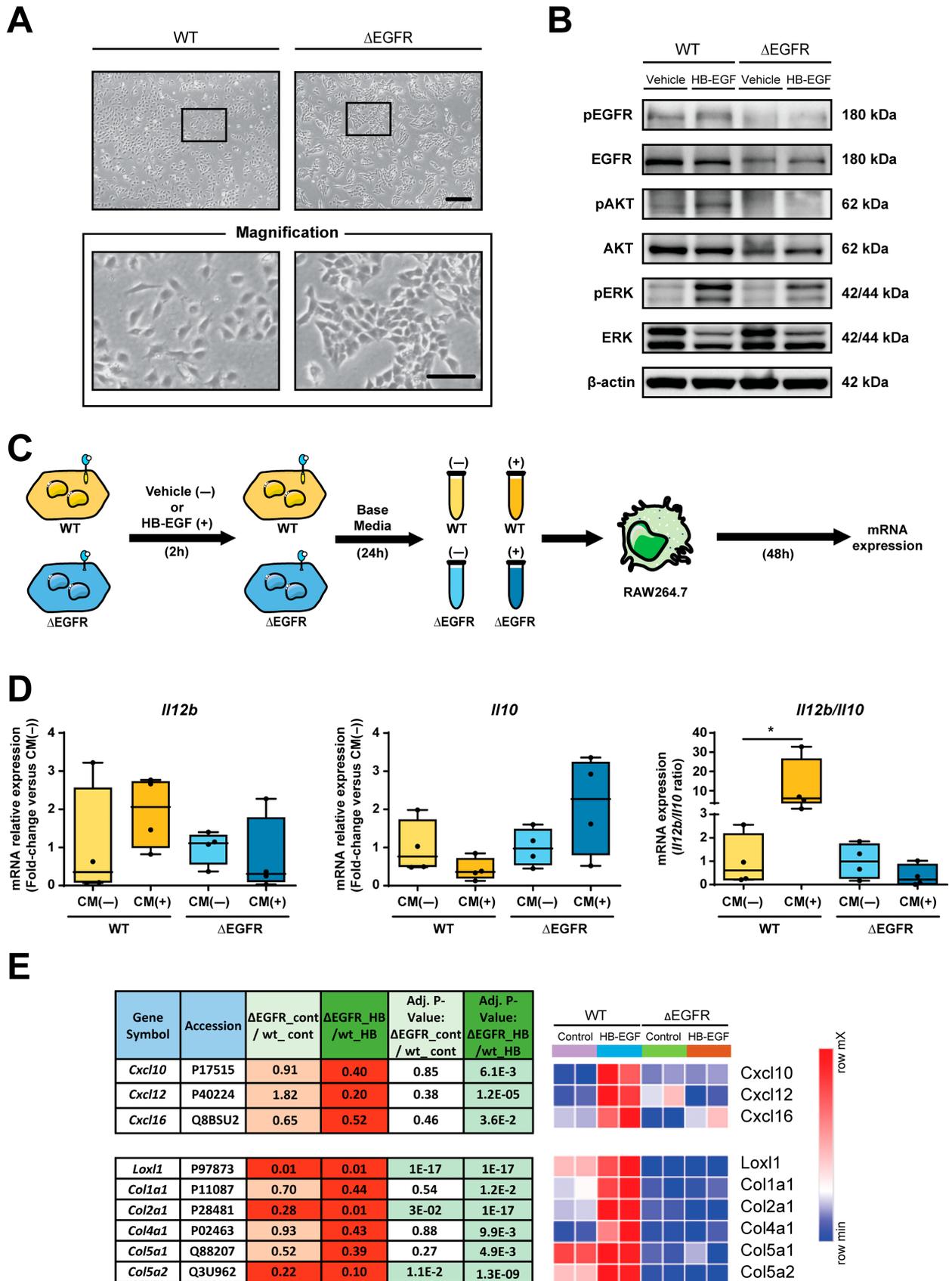


Figure 5. The EGFR pathway regulates the hepatocyte secretome. Effects of conditioned medium (CM) from WT and Δ EGFR hepatocytes untreated (-) or treated with HB-EGF (+) on the macrophage cell line (RAW264.7) phenotype. (A) Hepatocyte microscopy images. (B) Western blotting for EGFR pathway components in HB-EGF-treated hepatocytes. (C) Experimental approach. (D) RT-qPCR expression of *I12b* (M1) and *I110* (M2) genes, and the *I12b/I110* ratio. (E) Changes in pro-inflammatory and pro-fibrotic mediators in the secretomes of CM(-) and CM(+) from WT and Δ EGFR hepatocytes. Left: table (four independent experiments). Right: representative heatmap. Student's *t*-test. **p* < 0.05. Scale bars: 200 μ m.

The EGFR pathway is activated during the progression of fibrosis in humans, correlating with the expression of specific fibrotic and inflammatory genes

To analyze the translational relevance of the results presented above, data from gene expression in a cohort of liver biopsies from HCC-naïve MASLD (metabolic dysfunction-associated steatotic liver disease, previously named NAFLD) patients were accessed through GEO accession number GSE193066 (see Supplementary materials and methods). We observed that the relative enrichment of EGF/EGFR signaling (WikiPathways) was significantly increased during progression of liver fibrosis (Figure 6A). The relative enrichment of EGFR target genes (using the Gene Transcription Regulation Database publicly available at https://www.gsea-msigdb.org/gsea/msigdb/geneset_page.jsp?geneSetName=EGFR_TARGET_GENES) also presented a tendency, almost significant, to increase at advanced stages of fibrosis. Interestingly, the expression of fibrosis-related genes, such as collagen genes (*COL1A1*, *COL3A1*, *COL4A1*, *COL5A1*), *LOXLI*, *MMP2*, *MMP9*, or *TIMP1* presented a significant positive correlation with the EGF/EGFR signaling pathway (Figure 6B). Regarding genes related to the immune system, it was relevant that *CXCL10*, which was found to be regulated by the EGFR pathway in the secretome analysis in hepatocytes (Figure 5E), showed increased expression at advanced stages of fibrosis (Figure 6C), and the expression of *CXCL10* and *CXCL12* correlated with the EGF/EGFR signaling pathway (Figure 6D). A differential pattern was also found in the expression of *CD4* and *CD8A* (supplementary material, Figure S11A), which correlated with the differential pattern of Cd8⁺ and Cd4⁺ lymphocytes found in the Δ EGFR livers after CCl₄ treatment (supplementary material, Figure S5). Furthermore, the expression of *CD8A* positively correlated with the EGF/EGFR signaling (supplementary material, Figure S11B).

Discussion

Development of liver fibrosis is frequently the consequence of chronic hepatocyte damage, HSC activation and proliferation, and changes in ECM production and accumulation [1]. This process is accompanied by amplified inflammatory responses, with recruitment and activation of macrophages to the site of injury to orchestrate the process of wound healing and tissue repair [17,18]. Unidentified fibrosis can evolve into more severe consequences over time, such as cirrhosis and liver cancer [19]. Improving the knowledge about the intercellular interactions in the fibrotic niche is necessary to better understand this process and uncover potential therapeutic targets. This work demonstrates that the EGFR pathway in hepatocytes is required for an efficient progression of the fibrotic process in response to

CCl₄ treatment in mice. Attenuation of the EGFR kinase activity in hepatocytes reduces the parenchymal liver damage and the extension of fibrotic areas, correlating with a decrease in the expression of *Col3a1* and *Loxli*, an increase in *Mmp9*, and a decrease in *Timp1*. Previous studies had supported a role for the EGFR signaling pathway in the initiation and development of liver fibrosis [20–22]. However, in most of these works, the attention was mainly focused on the role of the EGFR pathway in hepatic steatosis and/or on HSC/MFB activation and proliferation. The main work that analyzed the role of the hepatocyte ERBB tyrosine kinases comes from Dr Russell's group, and suggested that EGFR–ERBB3 heterodimeric signaling in damaged hepatocytes may play a relevant role in liver fibrosis [9]. Our study constitutes a step forward for deepening insight into the molecular mechanisms that are regulated by the EGFR in hepatocytes during chronic liver damage.

The results shown here strongly indicate that after CCl₄ treatment, Δ EGFR livers present significant differences in the immune landscape, favoring the anti-inflammatory (M2) phenotype in macrophages. Despite tremendous progress, numerous challenges remain in deciphering the full spectrum of macrophage activation and its implication in either promoting liver disease progression or repairing injured liver tissue [23]. While in homeostatic conditions resident macrophages can be identified in the liver, in recent years numerous studies have provided compelling evidence that in inflammatory conditions there is considerably more heterogeneity among hepatic macrophages [24,25]. Experimental animal models indicate that macrophages are critical for fibrosis regression because they play key roles in repairing the necrotic lesions, not only by removing necrotic tissues but also by inducing cell death-resistant hepatocytes and exerting anti-inflammatory actions [26,27]. Although the role of M2-like macrophages in liver fibrosis is still controversial, strong evidence supports the theory that they could exert protection against injury, among other actions, by inhibiting necroptosis and conferring apoptosis resistance to hepatocytes [28,29]. Furthermore, M2 macrophage-derived exosomes significantly inhibit activation of HSCs [30]. The EGFR catalytic activity in hepatocytes also provoked differences in the T-cell populations, with an increase in the Treg lymphocytes and differences in the proportion of Cd8⁺ and Cd4⁺ cells, decreasing the naïve and IL17⁺ cells. It has been proposed that by inhibiting pro-fibrotic Th17 and Cd8⁺ T cells, Treg cells may help to maintain a healthy inflammatory environment, regulating the aberrant activation and functions of immune effector cells [31]. Overall, our results strongly indicate that the activation of the EGFR pathway in hepatocytes during chronic liver injury may have consequences on the inflammatory environment, regulating macrophage plasticity and favoring a high Th17/Treg cell ratio.

Analysis of the transcriptional signature of hepatocytes from fibrotic WT and Δ EGFR livers revealed the role of the EGFR pathway in regulating the expression of genes that promote fibrogenesis, such as different

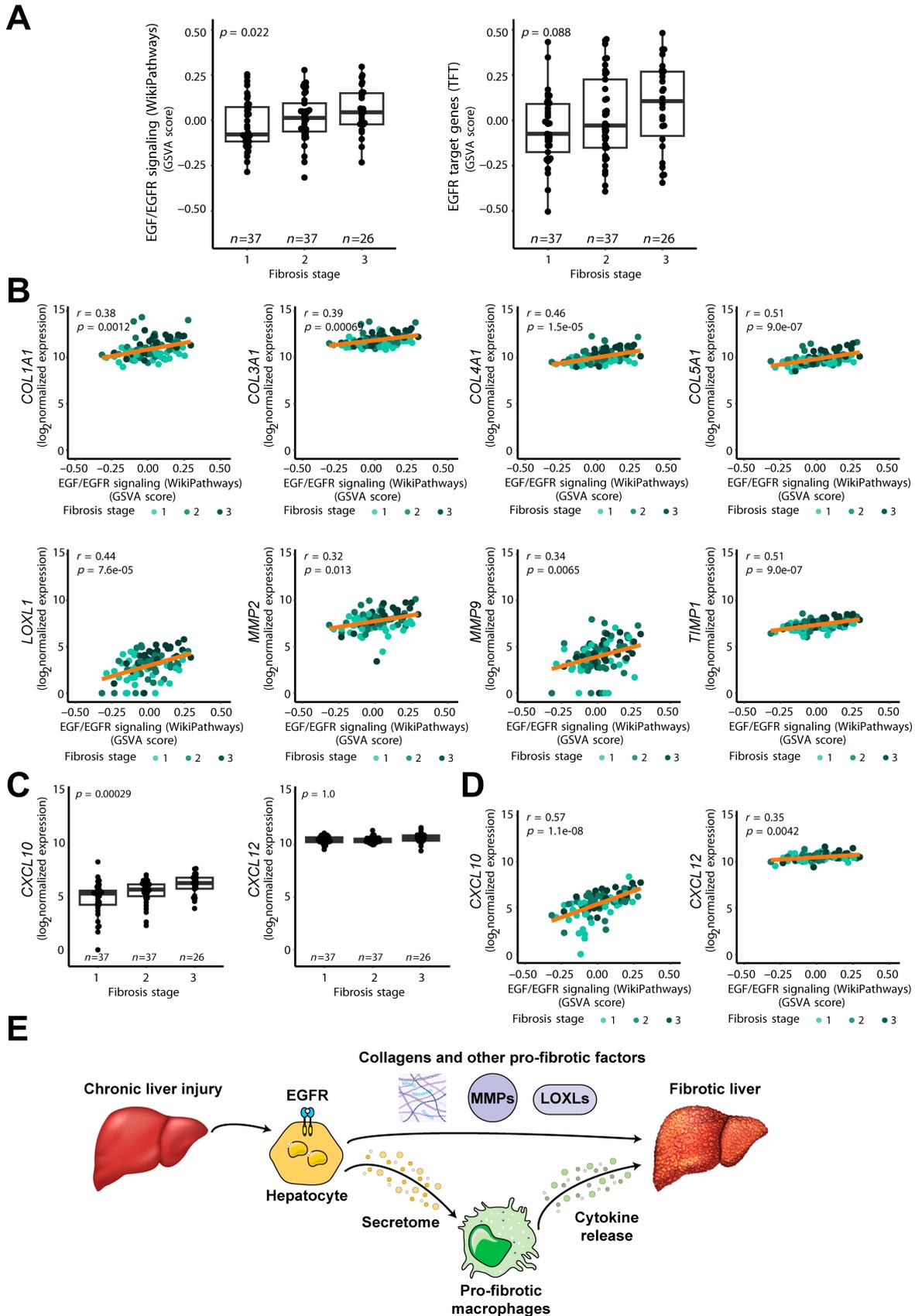


Figure 6. The EGFR pathway is activated in human fibrosis. (A) Boxplots of relative enrichment (GSEA score) of EGF/EGFR signaling and EGFR target genes across the fibrosis stages. (B) Pearson correlation of fibrosis-related genes' expression with EGF/EGFR signaling relative enrichment. (C) Boxplots of *CXCL10*/*CXCL12* across the fibrosis stages. (D) Pearson correlations between *CXCL10*/*CXCL12* gene expression and the EGF/EGFR signaling relative enrichment. Each dot is a sample (color indicates the fibrosis stage). (E) Schematic diagram depicting the major findings of the study. Created with [BioRender.com](https://www.biorender.com). Data were analyzed using Kendall's tau test (p values were adjusted for multiple testing).

mRNA forms of the *Pdgf* gene, as well as genes such as *Lox* and *Loxl1* coding for lysyl oxidases involved in collagen chain trimerization. Different genes related to inflammatory processes, such as genes related to the TNF pathway (*Tnfrsf12*, *Tnfrf11a*, *Tnfsfm13*), the pro-inflammatory dermokine (*Dmkn*), or regulators of the T-cell-mediated response, such as *Cd276*, also appeared upregulated specifically in WT livers from CCl₄-treated mice. Overall, the EGFR pathway modulates a transcriptomic program in hepatocytes that contributes to the generation of a pro-inflammatory and fibrotic environment, which justifies the strong differences observed between WT and Δ EGFR mice after CCl₄ treatment. Correlating with this, numerous TFs appeared regulated in CCl₄-treated WT but not in Δ EGFR hepatocytes. An aspect that caught our attention was the strong difference in the expression of genes related to proliferation and differentiation, which were upregulated and downregulated, respectively, in WT but not in Δ EGFR hepatocytes after CCl₄ treatment. Transient hepatocellular dedifferentiation can occur as part of the regenerative mechanisms triggered in response to acute liver injury. However, persistent downregulation of key identity genes is now accepted as a strong determinant of organ dysfunction in chronic liver disease [32], by stably reprogramming the critical balance of transcription factors responsible for hepatocyte identity, among them, HNF4 α [32,33]. Hepatocytes from CCl₄-treated Δ EGFR hepatocytes maintained *Hnf4a* expression and did not show changes in the expression of proliferation–differentiation genes, which may suggest that damage to hepatocytes is much less and/or that the EGFR pathway is required for this gene transcription reprogramming. In parallel, Δ EGFR hepatocytes displayed higher expression of genes related to mitochondrial metabolism pathways, which might confer greater efficient energy production capacity.

Our analysis of the secretomes of WT and Δ EGFR hepatocytes, either untreated or treated with HB-EGF, demonstrated that activation of the EGFR pathway alters the hepatocyte secretome, conferring the capacity to modulate macrophage polarity, promoting an anti-inflammatory phenotype. Proteomic analysis of the conditioned media revealed that the secretion of chemokines, such as CXCL10, CXCL12, or CXCL16, was significantly increased by HB-EGF in WT hepatocytes but not in Δ EGFR hepatocytes. These cytokines have been previously related to the progression of chronic liver injury. Intrahepatic CXCL10 has been strongly associated with liver fibrosis in different contexts in human liver pathologies [34,35], through regulation of macrophage-associated inflammation [36]. CXCL12, the ligand of CXCR4, can be involved in multiple pathological mechanisms in fibrosis, such as inflammation, immunity, EMT, and angiogenesis [37]. Furthermore, our secretome analyses indicated a role for hepatocytes in producing ECM proteins under the control of the EGFR pathway. It is worth highlighting that *Loxl1* appeared in all the studies presented in this paper: in the *in vivo* experiments, in the transcriptomic analysis performed in

hepatocytes, as well as in the secretome. It is very clear that hepatocytes contribute to the expression, production, and secretion of lysyl oxidases required for the final formation of the collagen fibers, and the EGFR pathway regulates this process.

Our data reveal that the findings obtained in the mouse model could be translated to human patients suffering from a fibrotic process. The analysis of transcriptomic data in a cohort of liver biopsies from HCC-naïve MASLD patients demonstrated that the EGFR signaling pathway increases in advanced stages of fibrosis and correlates with the expression of fibrotic and inflammatory genes identified in the *in vivo* and *in vitro* experiments. Expression of *LOXLI*, mentioned above as a clear candidate to be under the control of the EGFR pathway, as well as *TIMP1*, which has also been identified in some of the experimental analyses in the mouse model, showed a strong correlation with the EGF/EGFR signaling pathway. Also, it is worth highlighting *CXCL10*, whose expression shows a strong increase in advanced stages of fibrosis, positively correlated with the EGF/EGFR signaling pathway. Altogether, data from the human samples may indicate that genes that play essential roles during liver fibrosis/inflammation may be under the control of the EGFR pathway.

In conclusion, the data presented here lead us to propose a model where the EGFR pathway in hepatocytes controls liver fibrosis and inflammation in response to a chronic insult on the liver, through remodeling the hepatocytes' gene transcriptome/secretome, and which has consequences on the deposition of ECM as well as on the immune microenvironment (Figure 6E).

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Author contributions statement

EG-S, JV, DC-D, NPF, EB, JA, JG-S, BM-M, MG, AE-C, AA, CC, SC-P, PV, AMV, AS, BH and IF were involved in data acquisition, analysis, and/or interpretation. EG-S, JV, AS, BH and IF were involved in conceptualization and study design. EG-S, JV, AA, AS, BH and IF wrote the manuscript. EG-S, JV, DC-D, NPF, EB, JA, JG-S, BM-M, MG, AE-C, AA, CC, SC-P, PV, AMV, AS, BH and IF reviewed and edited the manuscript. JV, AMV, AS, BH and IF acquired funding. IF coordinated the work. All the authors reviewed and approved the final version of the manuscript.

Data availability statement

Raw and processed data from the RNA-seq analysis in hepatocytes have been submitted to NCBI's Gene Expression Omnibus (GEO) and are publicly available with GEO accession GSE265969 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE265969>). Other data are available on request from the corresponding author (IF).

References

- Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 151–166.
- Fabregat I, Caballero-Díaz D. Transforming growth factor- β -induced cell plasticity in liver fibrosis and hepatocarcinogenesis. *Front Oncol* 2018; **8**: 357.
- Bhushan B, Michalopoulos GK. Role of epidermal growth factor receptor in liver injury and lipid metabolism: emerging new roles for an old receptor. *Chem Biol Interact* 2020; **324**: 109090.
- Berasain C, Avila MA. The EGFR signalling system in the liver: from hepatoprotection to hepatocarcinogenesis. *J Gastroenterol* 2014; **49**: 9–23.
- Bhushan B, Banerjee S, Paranjpe S, et al. Pharmacologic inhibition of epidermal growth factor receptor suppresses nonalcoholic fatty liver disease in a murine fast-food diet model. *Hepatology* 2019; **70**: 1546–1563.
- Fuchs BC, Hoshida Y, Fujii T, et al. Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. *Hepatology* 2014; **59**: 1577–1590.
- Liang D, Chen H, Zhao L, et al. Inhibition of EGFR attenuates fibrosis and stellate cell activation in diet-induced model of nonalcoholic fatty liver disease. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 133–142.
- Perugorria MJ, Latasa MU, Nicou A, et al. The epidermal growth factor receptor ligand amphiregulin participates in the development of mouse liver fibrosis. *Hepatology* 2008; **48**: 1251–1261.
- Scheving LA, Zhang X, Threadgill DW, et al. Hepatocyte ERBB3 and EGFR are required for maximal CCl₄-induced liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2016; **311**: G807–G816.
- Santamaría E, Rodríguez-Ortígoza CM, Uriarte I, et al. The epidermal growth factor receptor ligand amphiregulin protects from cholestatic liver injury and regulates bile acids synthesis. *Hepatology* 2019; **69**: 1632–1647.
- Svinka J, Pflügler S, Mair M, et al. Epidermal growth factor signaling protects from cholestatic liver injury and fibrosis. *J Mol Med (Berl)* 2017; **95**: 109–117.
- López-Luque J, Caballero-Díaz D, Martínez-Palacián A, et al. Dissecting the role of epidermal growth factor receptor catalytic activity during liver regeneration and hepatocarcinogenesis. *Hepatology* 2016; **63**: 604–619.
- Lazcanoiturburu N, García-Sáez J, González-Corrales C, et al. Lack of EGFR catalytic activity in hepatocytes improves liver regeneration following DDC-induced cholestatic injury by promoting a pro-restorative inflammatory response. *J Pathol* 2022; **258**: 312–324.
- Berasain C, Nicou A, Garcia-Irigoyen O, et al. Epidermal growth factor receptor signaling in hepatocellular carcinoma: inflammatory activation and a new intracellular regulatory mechanism. *Dig Dis* 2012; **30**: 524–531.
- Sagmeister S, Drucker C, Losert A, et al. HB-EGF is a paracrine growth stimulator for early tumor prestages in inflammation-associated hepatocarcinogenesis. *J Hepatol* 2008; **49**: 955–964.
- Knockaert L, Berson A, Ribault C, et al. Carbon tetrachloride-mediated lipid peroxidation induces early mitochondrial alterations in mouse liver. *Lab Invest* 2012; **92**: 396–410.
- Gao CC, Bai J, Han H, et al. The versatility of macrophage heterogeneity in liver fibrosis. *Front Immunol* 2022; **13**: 968879.
- Hammerich L, Tacke F. Hepatic inflammatory responses in liver fibrosis. *Nat Rev Gastroenterol Hepatol* 2023; **20**: 633–646.
- Dhar D, Baglieri J, Kisseleva T, et al. Mechanisms of liver fibrosis and its role in liver cancer. *Exp Biol Med (Maywood)* 2020; **245**: 96–108.
- Chen L, Guo P, Li W, et al. Perturbation of specific signaling pathways is involved in initiation of mouse liver fibrosis. *Hepatology* 2021; **73**: 1551–1569.
- Choung S, Kim JM, Joung KH, et al. Epidermal growth factor receptor inhibition attenuates non-alcoholic fatty liver disease in diet-induced obese mice. *PLoS One* 2019; **14**: e0210828.
- Xu H, Liu L, Cong M, et al. EGF neutralization antibodies attenuate liver fibrosis by inhibiting myofibroblast proliferation in bile duct ligation mice. *Histochem Cell Biol* 2020; **154**: 107–116.
- Guillot A, Tacke F. Liver macrophages: old dogmas and new insights. *Hepatol Commun* 2019; **3**: 730–743.
- Guilliams M, Scott CL. Liver macrophages in health and disease. *Immunity* 2022; **55**: 1515–1529.
- Wang Z, Du K, Jin N, et al. Macrophage in liver fibrosis: identities and mechanisms. *Int Immunopharmacol* 2023; **120**: 110357.
- Feng D, Xiang X, Guan Y, et al. Monocyte-derived macrophages orchestrate multiple cell-type interactions to repair necrotic liver lesions in disease models. *J Clin Invest* 2023; **133**: e166954.
- Li W, He F. Infusion of Kupffer cells expanded *in vitro* ameliorated liver fibrosis in a murine model of liver injury. *Cell Transplant* 2021; **30**: 9636897211004090.
- Bai L, Kong M, Duan Z, et al. M2-like macrophages exert hepatoprotection in acute-on-chronic liver failure through inhibiting necroptosis-S100A9-necroinflammation axis. *Cell Death Dis* 2021; **12**: 93.
- Bai L, Liu X, Zheng Q, et al. M2-like macrophages in the fibrotic liver protect mice against lethal insults through conferring apoptosis resistance to hepatocytes. *Sci Rep* 2017; **7**: 10518.
- Wan Z, Yang X, Liu X, et al. M2 macrophage-derived exosomal microRNA-411-5p impedes the activation of hepatic stellate cells by targeting CAMSAP1 in NASH model. *iScience* 2022; **25**: 104597.

31. Ikeno Y, Ohara D, Takeuchi Y, *et al.* Foxp3+ regulatory T cells inhibit CCl₄-induced liver inflammation and fibrosis by regulating tissue cellular immunity. *Front Immunol* 2020; **11**: 584048.
 32. Berasain C, Arechederra M, Argemi J, *et al.* Loss of liver function in chronic liver disease: an identity crisis. *J Hepatol* 2023; **78**: 401–414.
 33. Loft A, Alfaro AJ, Schmidt SF, *et al.* Liver-fibrosis-activated transcriptional networks govern hepatocyte reprogramming and intra-hepatic communication. *Cell Metab* 2021; **33**: 1685–1700.e9.
 34. Brandt EF, Baues M, Wirtz TH, *et al.* Chemokine CXCL10 modulates the tumor microenvironment of fibrosis-associated hepatocellular carcinoma. *Int J Mol Sci* 2022; **23**: 8112.
 35. Singh KP, Zerbato JM, Zhao W, *et al.* Intrahepatic CXCL10 is strongly associated with liver fibrosis in HIV-hepatitis B co-infection. *PLoS Pathog* 2020; **16**: e1008744.
 36. Tomita K, Freeman BL, Bronk SF, *et al.* CXCL10-mediates macrophage, but not other innate immune cells-associated inflammation in murine nonalcoholic steatohepatitis. *Sci Rep* 2016; **6**: 28786.
 37. Wu X, Qian L, Zhao H, *et al.* CXCL12/CXCR4: an amazing challenge and opportunity in the fight against fibrosis. *Ageing Res Rev* 2023; **83**: 101809.
 38. Kao CY, Factor VM, Thorgeirsson SS. Reduced growth capacity of hepatocytes from c-myc and c-myc/TGF- α transgenic mice in primary culture. *Biochem Biophys Res Commun* 1996; **222**: 64–70.
 39. Dobin A, Davis CA, Schlesinger F, *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013; **29**: 15–21.
 40. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 2011; **12**: 323.
 41. Law CW, Chen Y, Shi W, *et al.* voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol* 2014; **15**: R29.
 42. Kolberg L, Raudvere U, Kuzmin I, *et al.* gprofiler2 – an R package for gene list functional enrichment analysis and namespace conversion toolset g:profiler. *F1000Res* 2020; **9**: ELXIR-709.
 43. Hänzelmann S, Castelo R, Guinney J. GSEA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformatics* 2013; **14**: 7.
 44. Vaquero J, Lobe C, Tahraoui S, *et al.* The IGF2/IR/IGF1R pathway in tumor cells and myofibroblasts mediates resistance to EGFR inhibition in cholangiocarcinoma. *Clin Cancer Res* 2018; **24**: 4282–4296.
 45. Liberzon A, Birger C, Thorvaldsdóttir H, *et al.* The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* 2015; **1**: 417–425.
 46. R Core Team. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing: Vienna, Austria, 2022. [Accessed 31 May 2024]. Available from: <https://www.R-project.org/>.
- References 38–46 are cited only in the supplementary material.

SUPPLEMENTARY MATERIAL ONLINE

Supplementary materials and methods

Figure S1. Experimental mouse model

Figure S2. Analysis of the transforming growth factor-beta (TGF- β) pathway in livers from CCl₄-treated Δ EGFR and WT mice

Figure S3. Expression of pro-fibrotic factors in livers from CCl₄-treated Δ EGFR and WT mice

Figure S4. Inflammatory status in livers from CCl₄-treated WT and Δ EGFR mice

Figure S5. Impairment of the EGFR catalytic activity in hepatocytes alters the lymphocytic infiltrate following CCl₄-induced liver damage

Figure S6. The EGFR pathway regulates the hepatocyte gene transcriptome in response to CCl₄

Figure S7. The EGFR pathway regulates the hepatocyte gene transcriptome during the response to CCl₄

Figure S8. The impairment of EGFR catalytic activity in hepatocytes does not significantly impact the TGF- β pathway during the response to CCl₄

Figure S9. The impairment of EGFR catalytic activity in hepatocytes does not significantly impact the EMT pathway during the response to CCl₄

Figure S10. The EGFR pathway regulates the human hepatocyte secretome

Figure S11. EGFR signaling correlates with changes in lymphocyte populations in human fibrosis

Table S1. Primary antibodies used for immunodetection (referred to in Supplementary materials and methods)

Table S2. Antibodies used for immunodetection of liver immune populations by FACS (referred to in Supplementary materials and methods)

Table S3. Primers used for RT-qPCR (mouse genes) (referred to in Supplementary materials and methods)

Table S4. Primers used for RT-qPCR (human genes) (referred to in Supplementary materials and methods)

Table S5. In-house EMT signature genes designed by Fabregat's laboratory