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COMMENTARY

Illuminating liver fibrosis with vitamin D

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Summary Hepatic fibrosis results from the accumulation of extracellular matrix-producing myofibroblasts in the liver. The mechanisms leading to the activation of hepatic stellate cells (HSCs) into myofibroblasts have been well described. By contrast, few molecular pathways leading to myofibroblast deactivation have been documented. Recently, the vitamin D-VDR axis has been shown to modulate HSC activity through a complex mechanism involving epigenetic modifications induced by the SMAD pathway.

Hepatic fibrosis is the unwanted but obligatory consequence of chronic liver diseases. Fibrous tissue deposition by disrupting hepatic architecture finally leads to cirrhosis and liver failure. The central players of extracellular matrix deposition are non-parenchymal liver cells. Two cell types are now well recognized as being involved in liver fibrosis, *i.e.* hepatic stellate cells (HSCs) and portal mesenchymal cells [1]. The molecular entities involved in myofibroblast activation have been extensively described [1], while molecules able to deactivate myofibroblasts are less known [2]. In a recent paper published in *Cell*, Ding et al. shed light on an epigenetic mechanism involving the vitamin D receptor (VDR) and SMAD signaling that leads to myofibroblast deactivation [3].

Ding et al. showed that calcipotriol, a low-calcemic vitamin D analog, decreases hepatic fibrosis in a mouse model of carbon tetrachloride (CCl_4) intoxication. In this model, hepatic fibrosis was induced by CCl_4 during twenty days, and mice were then co-treated with calcipotriol for eight days. The antifibrotic effects of calcipotriol were evidenced by the reduction of histological fibrotic score, collagen deposition and expression of fibrotic marker genes (*i.e. Col1a1*, *Tgfβ1* and *Timp1*). Furthermore, pretreatment of CCl_4-injected mice by calcipotriol for five weeks also prevented the development of liver fibrosis. Reciprocally, 6-month-old *Vdr*$^{-/-}$ mice exhibited extensive spontaneous liver fibrosis associated with necrosis and portal tract inflammation.

The authors next explored the impact of vitamin D on the expression profile of HSCs. 1,25-dihydroxyvitamin D_3 (*i.e.* $1,25(OH)_2D_3$) repressed 39 fibrotic marker genes that were induced by TGFβ1 in primary rat HSCs. The effect of either $1,25(OH)_2D_3$ or calcipotriol was inhibited when the expression of VDR was abolished, suggesting that vitamin D decreases hepatic fibrosis by limiting HSC activation through an anti-TGFβ1 effect mediated by VDR.

Because VDR activation did not directly impact the TGFβ1 signaling pathway, the authors propose a direct regulating role for VDR on HSC biology. Analysis of the genome-

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wide binding sites of VDR and SMAD3 (*i.e.* cistromes) in the HSC cell line LX-2 revealed 11,031 and 9,210 putative target genes, respectively. Interestingly, the binding sites for VDR and SMAD3 were respectively co-enriched within nucleosomal distance with SMAD3 and VDR binding motifs, suggesting that VDR and SMAD3 may interact through overlapping cistromes. Indeed, bioinformatic analyses indicated that 10,436 genomic sites were co-occupied by both VDR and SMAD3. The latter observation was confirmed by ChIP-on-ChIP experiments that showed that VDR and SMAD3 do occupy the same genomic binding sites. Among the 39 profibrotic genes regulated by both VDR ligands and TGFβ1 in HSCs, 34 genes contain at least one VDR/SMAD3 binding site, suggesting an interplay between the two transcription factors in HSCs.

The fact that VDR and SMAD3 DNA binding sites were in close proximity suggests the existence of a genomic crosstalk between these two transcription factors. Indeed, VDR binding was enhanced by an order of magnitude in HSCs treated with TGFβ1, while SMAD3 binding was decreased by approximately 1.5-fold by calcipotriol. Thus, TGFβ1 promotes binding of liganded VDR to *cis*-regulatory regions of profibrotic genes.

Analysis of the VDR cistrome revealed 6,281 binding sites under calcipotriol treatment, while 24,984 binding sites were observed under calcipotriol and TGFβ1 co-treatment. These results indicate that TGFβ induces a dramatic shift in genome-wide binding locations of liganded VDR. Local chromatin remodeling is accountable for disclosing these VDR binding sites, as histone H3 hyperacetylation decreased in the presence of TGFβ and calcipotriol. Finally, VDR and SMAD3 DNA binding were inversely correlated in the *cis*-regulating regions of profibrotic genes, such as *Col1a1*, suggesting that liganded VDR antagonizes SMAD3 binding to repress profibrotic gene expression. In conclusion, the study by Ding et al. suggests that TGFβ enhances VDR binding ability to genomic sequences in HSCs resulting in profibrotic pathway inhibition (Fig. 1).

Ding et al. suggest that transcription factor crosstalk may be a key mechanism in liver fibrosis resolution. Among transcription factors, nuclear receptors, such as the peroxisome proliferator-activated receptor gamma (PPARγ), the liver X receptor beta (LXRβ) the farnesoid X receptor (FXR), have already been shown to be involved in liver fibrosis through the control of HSC activation. The expression of PPARγ, a central player of the quiescent HSC phenotype, is decreased in activated HSCs [4]. Furthermore, PPARγ inhibits human HSC activation *in vitro* [5], and is able to repress the expression of profibrotic markers in activated HSCs [6]. Consistently, administration of PPARγ ligand decreases fibrosis by limiting collagen deposition in rats [4,7]. LXRβ may also modulate HSC activation by limiting pro-inflammatory mediator secretion. Indeed, LXRs ligands suppress the expression of profibrotic markers *in vitro*, while deletion of LXRα/β increases the fibrogenic response of HSCs to exacerbate liver fibrosis *in vivo* [8]. Activation of FXR has also been shown to limit liver fibrosis in the rat by modulating HSC activity by a SHP-dependent mechanism [9]. However, FXR expression was not observed in mouse and human HSCs [8,10], suggesting a minimal role in HSC control. By contrast, VDR is highly expressed in HSCs when compared to other nuclear receptors [10], and $1,25(OH)_2D_3$ limits hepatic fibrosis in TAA-treated rats by inhibiting HSC activation [11].

VDR conveys the biological effects of vitamin D by forming a heterodimer with RXR that binds to DNA to control expression of target genes [12,13]. Accordingly, VDR expression has been identified in all major vitamin D target tissues, such as bone, kidney, thyroid and intestine [14]. The liver is usually described as negative for VDR expression [14], even though HSCs and other non-parenchymal liver cells express VDR [15,16]. The latter observation suggests that VDR has the potential to impact hepatic processes involving non-parenchymal cells, such as liver fibrosis [17].

Consistently, VDR polymorphisms have been identified in primary biliary cirrhosis, a disease targeting biliary epithelial cells and characterized by focal biliary fibrosis [18–22]. Furthermore, low vitamin D serum levels have

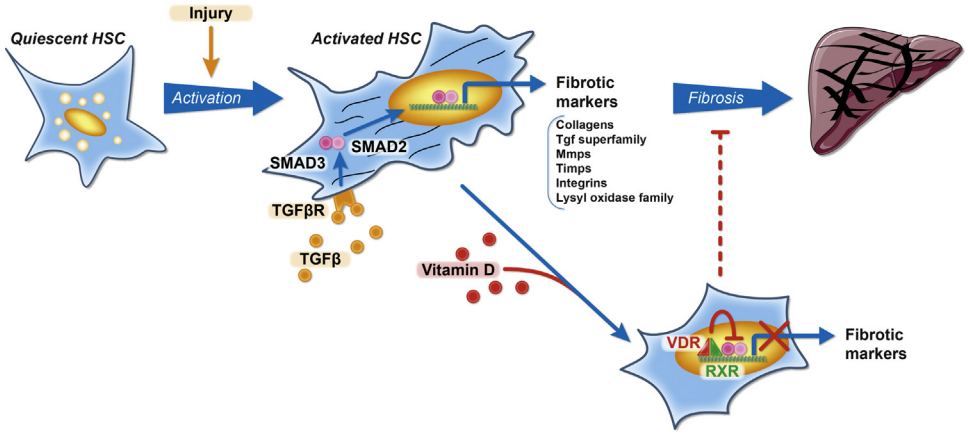

Figure 1 Regulation of hepatic fibrogenesis by a VDR/SMAD crosstalk. In response to liver injury, quiescent hepatic stellate cells (HSCs) are activated, thus exhibiting a profibrotic phenotype. Profibrotic gene expression is then induced by transforming growth factor β (TGFβ) signaling pathway, through its downstream effectors SMAD2 and SMAD3. The study by Ding *et al.* demonstrates that activated vitamin D receptor (VDR) antagonizes SMAD2/3 DNA binding to repress profibrotic gene expression. Mmps: matrix metalloproteinases; RXR: retinoid X receptor; TGFβR: TGFβ receptor; Timps: tissue inhibitors of metalloproteinases.

been correlated with the extent of inflammation and fibrosis in the setting of viral hepatitis C [23—25]. However, the cellular and molecular mechanisms linking the vitamin D-VDR axis to liver fibrosis have long remained elusive. In the kidney, interstitial fibrosis induced by unilateral ureteral obstruction can be decreased by paricalcitol, a vitamin D analog [26]. Paricalcitol inhibits kidney fibrosis by limiting the induction of epithelial to mesenchymal transition (EMT) of tubular epithelial cells by TGFβ [26], thus suggesting that vitamin D may prevent organ fibrosis by limiting EMT [27]. The possibility that liver epithelial cells undergo EMT to induce fibrosis originate from following the observation that periportal fibrosis arises following the loss of biliary epithelial cells in primary biliary cirrhosis [28,29]. However, cell-tracing methods have shown that biliary epithelial cells do not undergo EMT in hepatic fibrosis [30,31]. Nonetheless, we have recently shown that VDR limits the ability of biliary epithelial cells to acquire features of EMT and thus protects the biliary epithelium from disruption arising from the loss of cell-cell contacts in the setting of biliary obstruction [32]. In primary biliary cirrhosis, the simultaneous activation of VDR and FXR in biliary epithelial cells may also be protective by enhancing innate immunity [33]. The latter observation suggests that VDR may impact liver disease leading to fibrosis by interacting with other transcription factors. This possibility is further supported by the observation that VDR crosstalks with other nuclear receptors [34,35] or with other classes of transcription factors, such as SMADs [36].

In vitro, the activity of liganded VDR is enhanced by SMAD3 through direct interaction [36]. Physical interaction of activated VDR with SMAD has also been shown to decrease TGFβ auto-induction by allowing pSMAD3 dephosphorylation in kidney epithelial cells [37]. Direct interaction may however only be observed when both transcription factors are bound to their respective response elements [38]. In line with this observation, Ding et al. show that VDR signaling is enhanced by SMAD3 by epigenetic modifications revealing cryptic VDR binding sites [3]. Epigenetic modifications constitute a set of regulatory mechanisms (*i.e.* DNA methylation, post-translational modifications of histones and non-coding RNAs) that are increasingly recognized as involved in hepatic fibrogenesis [39]. As an example, HSCs acquire profibrogenic phenotype when PPARγ expression is decreased by an epigenetic mechanism involving the control of histone methylation by miRNA-132 [40]. Because the vitamin D-VDR axis is known to control the expression of various miRNA in epithelial cells [41,42], VDR has also the potential to control liver fibrosis by epigenetic mechanisms. Indeed, Ding et al. demonstrate that the vitamin D-VDR axis controls hepatic fibrosis through an epigenetic mechanism that involves a VDR/SMAD genomic circuit including chromatin remodeling and histone acetylation [3].

In conclusion, Ding et al. shed light on a new molecular mechanism regulating liver fibrosis that involves a crosstalk between transcription factors through epigenetic modifications. Even though the demonstration of this specific crosstalk in HSCs may be difficult to ascertain *in vivo*, this mechanism should be confirmed in other *in vivo* models of liver fibrosis, such as bile duct ligation or diethylnitrosamine intoxication. Nevertheless, the observation of Ding et al. opens new exciting fields of research and already suggests that epigenetic modifications and the vitamin D-VDR axis may be targeted in hepatic fibrosis.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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