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A slice of life – Dissecting cell-specific function of PPARγ in liver and embracing complexity

A lovely study in this month's issue of the *Journal* provides a nice example of using complementary approaches to dissect cell-specific function in an animal model of liver injury. The study by Morán-Salvador *et al.* examines the contributions of PPARγ activity in both parenchymal and non-parenchymal cells during CCl_4-induced liver injury by combining a method to genetically deplete this gene in specific cell types *in vivo* with a model of tissue function using precision cut liver slices (PCLS) *ex vivo.*

PPARγ (peroxisome proliferator activated receptor gamma) is a well characterized nuclear transcription factor that is one of three subtypes of this receptor family, which also includes PPARs α and β/δ [1]. The receptors always pair with the Retinoid X receptor (RXR) to form obligate heterodimers in regulating gene expression, and, like other nuclear receptors, they engage specific ligands in the cytoplasm, then translocate to the nucleus where they regulate gene targets by binding to discrete DNA promoter elements. PPARγ has two isoforms generated through alternative splicing, PPARγ1 and PPARγ2, each with a distinct tissue distribution and repertoire of target genes; in the Morán-Salvador study, changes in their model were largely restricted to PPARγ1. Although only a few endogenous ligands for PPARγ have been identified, the discovery of thiazolidinedinones (TZDs) as synthetic PPARγ activators that sensitize insulin signaling has led to their widespread use as antidiabetic agents. More recently, however, enthusiasm for using them has waned because of concerns about increased adverse cardiac events, as well as edema and weight gain associated with these agents (e.g., rosiglitazone or Avandia®, and pioglitazone or Actos®) [2]. The concerns notwithstanding, the discovery of these first generation synthetic PPARγ ligands has been instrumental in clarifying PPARγ's mechanisms of action in regulating lipid homeostasis, inflammatory signaling and glucose regulation.

Given PPARγ's very broad range of potential activities and off-target effects, it has been essential to define both its tissue-specific and cell-specific effects. The molecule contributes significantly, but sometimes divergently to a range of pathways in adipose, skeletal muscle, immune cells, bone and brain, among others [1]. In liver, clarifying the cell-specific effects is especially pertinent given the important contributions of dyslipidemia, inflammation and insulin resistance to the rising prevalence of non-alcoholic steatohepatitis (NASH). Yet, to date, the effects attributed to PPARγ in liver are uncertain and sometimes contradictory. The authors of the current manuscript and others have previously suggested that PPARγ agonism promotes steatosis through enhanced lipid uptake by hepatocytes [3–5], whereas at least one study has demonstrated the opposite effect [6]. In stellate cells, the key fibrogenic cell in liver, studies have consistently reported that increased PPARγ signaling attenuates cellular activation and is antifibrotic [7–9]. In inflammatory cells including macrophages, dendritic cells and lymphocytes, different subsets each respond uniquely to PPARγ signaling [1].

How does one tease out the specific cellular responses to a broadly expressed signaling pathway in a complex tissue? The development of cell-specific knockout technology in experimental mouse models offers an ingenious solution. In this widely exploited technique, a mouse is generated in which a gene locus of interest, in this case PPARγ, is genetically altered so that the locus is flanked in every cell type by a target sequence known as LoxP, which is recognized specifically by an exogenous bacteriophage enzyme known as Cre recombinase. If constructed properly, these mice with 'floxed alleles' (i.e., flanked by LoxP) have normal gene function. However, when Cre is expressed in specific tissues, Cre-Lox-mediated recombination occurs, effectively replacing the functioning gene with one that is inactivated. To achieve cell-specific gene knockout, mice with these floxed alleles can be mated with another mouse line in which Cre expression only occurs in those cells where the promoter that drives Cre is active [10].

This is exactly the strategy used by Morán-Salvador and colleagues to dissect the relative contributions of PPARγ expressed by hepatocytes, macrophages, and stellate cells to CCl_4 liver injury. They crossed the same PPARγ floxed mouse line with three different Cre-expressing mouse lines, one expressing albumin-Cre (Alb-Cre) for hepatocytes, lysozyme M-Cre (LysM-Cre) for inflammatory cells, and adipocyte fatty acid-binding protein-Cre (aP2-Cre) for hepatic stellate cells. Their results clearly indicate that deletion of PPARγ in inflammatory cells, and to a lesser extent in hepatocytes, significantly worsened CCl_4 liver

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injury, lipid peroxidation and death of hepatocytes. Increased injury was associated both with significant elevations in isolated macrophages of several inflammatory mediators, including COX-2, TNF−α, CXCL2 and IL-1β, and with increased fibrosis *in vivo*. Similarly, deletion of PPARγ in stellate cells by expression of aP2-Cre led to amplified CCl_4 liver injury and fibrosis. These data support the conclusion that PPARγ normally suppresses inflammation and injury, whereas its deletion amplifies these features.

While these *in vivo* findings evince the power of cell-specific gene deletion to clarify disease pathogenesis, they do not exclude the possible contribution from cells expressing Cre outside the liver, since both LysM-Cre and aP2-Cre gene expression is not restricted to the liver, with LysM expressed in all macrophages and neutrophils, and aP2-Cre also expressed in adipocytes. This is an important concern in view of the prevailing evidence that cross talk between liver and other organs (especially adipose) mediates some features of NASH [11]. In other words, did circulating cells derived from adipose or elsewhere account for the enhanced inflammation in liver following PPARγ deletion?

To exclude the impact of circulating factors from other organs on the liver phenotype, the authors used precision cut liver slices (PCLS), a well established technique that has the advantage of preserving cells in their native context while eliminating the effect of stimuli coming from outside the liver. PCLS are an excellent tool for short-term studies in which cell-cell interactions must be maintained [12,13], although the technique has not yet been widely exploited in the field yet. In the current study, the authors examined the effects of lipopolysaccharide (LPS), a well-known macrophage activator, on PCLS from LysM-Cre/PPARγ floxed mice, demonstrating that these tissue slices were prone to greater levels of liver injury. In support of a protective role of PPARγ, when PCLS from normal mice were incubated with LPS, injury was attenuated in the presence of rosiglitazone. Interestingly, in PCLS from Alb-Cre/PPARγ floxed mice, there was no increased injury following LPS, suggesting that the increased hepatocyte injury seen *in vivo* could indeed be due to infiltration of circulating inflammatory cells, since these are not present in the PCLS model.

The study by Morán-Salvador *et al.* is not perfect, however. Only one model of liver injury was used, and the LysM-Cre transgene is also expressed in neutrophils. Thus, the role of PPARγ could be restricted to injury due to CCl_4 (although unlikely), and the important contribution of neutrophils to the phenotype [14] was not quantified. Moreover, it is unclear which subsets of macrophages express the LysM promoter, even though different subsets could elicit very disparate effects [15]. Also, PCLS were not analyzed from aP2-Cre floxed mice, and since the aP2 promoter is active in adipocytes, a role of this cell type in contributing to the liver phenotype after CCl_4 in aP2-Cre/PPARγ floxed mice cannot be excluded.

Do these findings have any clinical implications? You bet they do! TZDs have been studied extensively in human liver disease trials both as agents either to attenuate NASH or to reduce fibrosis in patients with chronic HCV, yet the results have not been impressive in either disease. Perhaps this is because of competing activities of the drugs in different cell types that undermine their efficacy, both within liver and among other tissues. In the case of NASH, it's fair to say that the effects of rosiglitazone and pioglitazone have been modest, at best. In a trial of rosiglitazone from France, a transient impact on steatosis was evident but there was no long-term benefit [16]. In the PIVENS trial supported by the NIDDK NASH Clinical Research Network, pioglitazone improved both steatosis and lobular inflammation but had no impact on fibrosis [17]. And finally, in a trial of an experimental TZD, farglitazar, the drug had no effect on stellate cell activation or fibrosis in chronic HCV patients [18].

With rising concerns about adverse effects of the current TZDs, a more targeted approach to inducing PPARγ signaling is necessary, which can only be framed around a clearer picture of how PPARγ activity is regulated in specific cell types at the levels of transcription, translation, epigenetics and post-translational modification. As nicely reviewed by Ahmadian and co-authors [1], newer strategies for selective PPARγ agonism might include the development of more selective ligands that only affect specific pathways or tissues, altering post-translational modification of PPARγ, using mixed PPAR receptor targeting, or regulating specific lipases to generate lipid ligands for PPARγ within precise cell types. Finally, greater clarification of PPARγ targets that mediate its effects, for example FGF1 and FGF21, could lead to efforts to modulate downstream effectors of PPARγ activity rather than the nuclear receptor itself.

In aggregate, the study by Morán-Salvador *et al.* exploits complementary models to help us understand and embrace the complexity of PPARγ signaling, which should lead us towards more specific and safer drugs to treat liver disease.

Conflict of interest

The author declared that he does not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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