

Fat paradox in liver disease

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Abstract. Alcoholic liver disease (ALD) is characterized by accumulation of neutral lipids in hepatocytes leading to micro and macro-vesicular steatosis and balloon cell degeneration. Hypercaloric alimentation and resultant obesity also cause similar changes as evident in non-alcoholic fatty liver disease (NAFLD). Thus, accumulation of lipids in hepatocytes is a pathologic hallmark of ALD and NAFLD. In contrast, quiescent hepatic stellate cells (HSC) are characterized by the intracellular content of not only vitamin A but also triglycerides, and HSC activation is associated with depletion of these lipids. In fact, our recent work demonstrates that adipogenic/lipogenic transcriptional regulation rendered by PPAR γ , LXR α , and SREBP-1c is essential for the maintenance of the fat-storing, quiescence phenotype of HSC. Expression of these adipogenic transcription factors is lost in activated HSC and the treatment of the cells with the adipocyte differentiation cocktail or ectopic expression of PPAR γ or SREBP-1c causes a reversal of activated cells to the quiescent phenotype. In steatotic livers from ALD and NAFLD mouse models, the expression of these adipogenic transcription factors is induced while the normal control livers lack such expression. Thus, adipogenic regulation is essential for HSC quiescence while it makes hepatocytes steatotic. Interestingly, under the adipogenic conditions of ALD and NAFLD, HSC are still activated to cause fibrosis. This fat paradox in hepatocytes and HSC highlights contrasted significance of fat in these two cell types that depend on each other for their homeostatic control. It further suggests, activated HSC in steatotic livers may have defective insulin signaling or lipogenic regulation. (Keio J Med 54 (4): 190–192, December 2005)

Key words: Ito cells, hepatic stellate cells, PPAR γ , adipocyte

PPAR γ as a Molecular Target of Anti-liver Fibrosis Modality

In 2000, three independent publications appeared that reported inhibition of activated hepatic stellate cells (HSC) with ligands for peroxisome proliferator-activated receptor γ (PPAR γ) *in vitro*.^{1,4,5} Our report also demonstrated that HSC activated both *in vitro* and *in vivo* have reduced expression of PPAR γ and that the treatment of culture-activated HSC with the PPAR γ ligands restores its expression.¹ These results raised a possibility that PPAR γ may serve as a therapeutic target for liver fibrosis. This notion was subsequently tested by Galli *et al*, who demonstrated the therapeutic efficacy of two thiazolidinedione (TZD) derivatives (pioglitazone and rosiglitazone) in two toxic and one cholestatic models of liver fibrosis.⁶ This study also

confirmed the depletion of PPAR γ in HSC nuclear extracts from fibrotic livers and a correction of this defect by the TZD treatment *in vivo*.

Ectopic Expression of PPAR γ in Culture-activated HSC

TZDs are known to have multiple biological effects including suppression of inflammation and cell proliferation, pro-apoptotic effects, besides the aforementioned anti-fibrotic effects. These effects may or may not be mediated via PPAR γ . A PPAR γ independent effect was unequivocally demonstrated by Chawla *et al*, who showed neither macrophage differentiation nor anti-inflammatory effects of TZD are abrogated in macrophages derived from PPAR γ null mouse embryonic stem cells.⁷ Thus, it is critical to determine whether

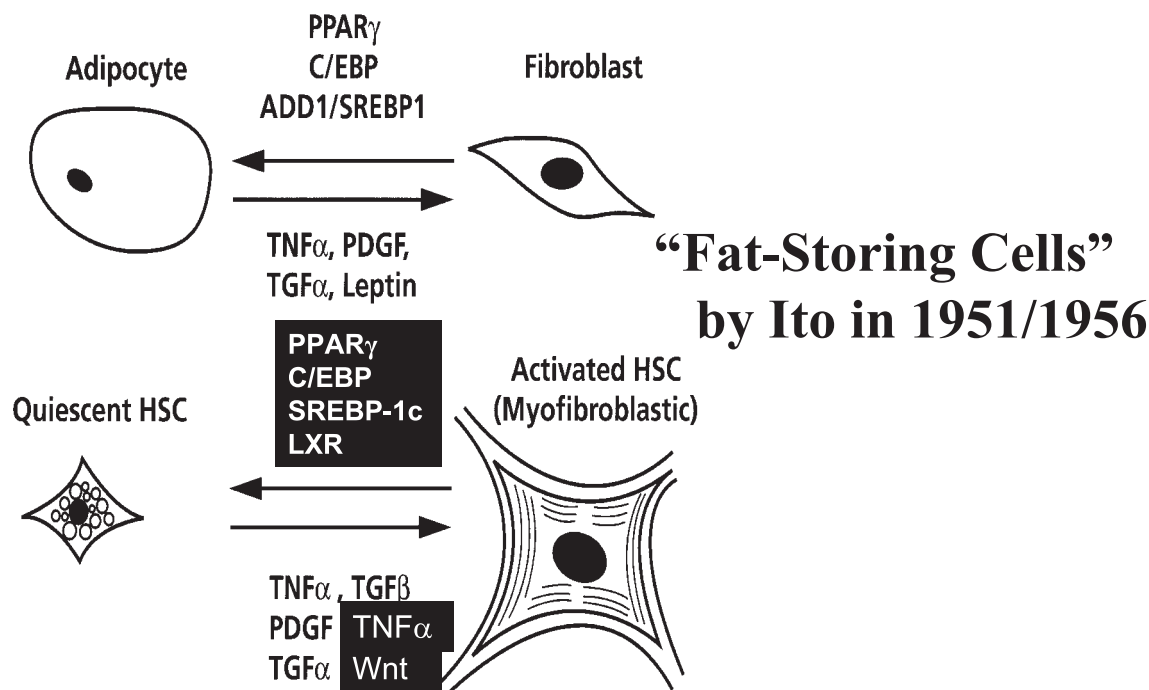


Fig. 1 A schematic diagram depicting analogy between preadipocyte-adipocyte transdifferentiation and HSC activation. As required for adipocyte differentiation, a group of transcription factors including C/EBPs, PPAR γ , LXR α , and SREBP-1 are essential for the maintenance of adipogenic, quiescent phenotype of HSC. HSC was once termed “Fettspeicherungszellen: fat-storing cells” by Professor T. Ito upon his characterization of the cells in 1951 and 1956 (see references 8 and 9). This fat-storing phenotype of HSC needs to be re-visited.

PPAR γ *per se* is important for the demonstrated anti-fibrotic effects. To this end, our laboratory used an adenoviral vector to ectopically express PPAR γ in culture-activated HSC.² This study demonstrated that expression of PPAR γ itself is sufficient to reverse the morphological and biochemical characteristics of activated HSC. In particular, PPAR γ -transduced cells are able to accumulate vitamin A in the presence of retinol and palmitate and this effect is blocked by co-infection with a vector expressing a dominant negative mutant of PPAR γ . As a mechanistic insight into the observed reversal, PPAR γ is shown to decrease the binding of JunD to the AP-1 site. This effect is not mediated by reduced expression of JunD or suppressed JNK activity. But it is due to JunD-PPAR γ interaction as demonstrated by co-immunoprecipitation and glutathione *S*-transferase pull-down analysis. Further, the use of deletion constructs revealed that the DNA binding region of PPAR γ is the JunD interaction domain.

Adipogenic Transcriptional Regulation in HSC

The above findings solidified the notion that PPAR γ serves as an important therapeutic target for liver fibrosis. They also supported a more fundamental notion that differentiation of HSC is similar to that of adipocytes. This notion is based on several known common-

alities. Firstly, both quiescent HSC and adipocytes store lipids while activated HSC and pre-adipocytic fibroblast are devoid of lipid storage. Secondly, both quiescent HSC and adipocytes express extracellular matrix proteins of the basement membrane while activated HSC and preadipocytes mainly produce interstitial collagens. Thirdly, soluble factors shown to activate HSC such as PDGF, TGF β , TNF α , leptin, etc. are also known mediators that inhibit adipocyte differentiation. Lastly, PPAR γ is now shown to serve as a key transcription factor for HSC quiescence as it is considered as a master regulator for adipogenesis. If HSC are similar to adipocytes, HSC quiescence should be facilitated by a group of adipogenic transcription factors besides PPAR γ . These factors include CREB, C/EBP α , β and δ , LXR α , and SREBP-1c. In fact, these factors and PPAR γ have positive-forward and cross-inductive regulation in order to orchestrate full transcriptional activation required for adipocyte differentiation. Thus, to test our notion of the similarity between HSC and adipocytes, we examined the expression of these putative adipogenic transcription factors in quiescent and culture-activated HSC. Indeed, quiescent and fully-differentiated HSC express C/EBP α and β , an active form of SREBP-1c, and LXR α and their levels rapidly decline as HSC become activated and lose their lipid content in culture.³ Coordinately, adipocyte-specific

genes that are mostly down stream of PPAR γ and SREBP-1c are expressed at the higher levels in quiescent HSC including adipon and resistin.

Gain of Adipogenic Transcriptional Factors Restores HSC Quiescence

To test the causal relationship between HSC trans-differentiation (activation) and the loss of adipogenic transcription factors, we have used three different approaches of gain of function: 1) the treatment of activated HSC with the adipocyte differentiation cocktail (insulin, dexamethasone, isobutylmethylxanthine); 2) ectopic expression of PPAR γ as previously performed by an adenoviral vector; and 3) virally mediated ectopic expression of SREBP-1c. In summary, all three manipulations reversed morphologic and biochemical characteristics of culture-activated HSC to quiescent HSC while restoring the expression of the adipogenic transcription factors.³ These results are in support of the central hypothesis that HSC quiescence is maintained by adipogenic transcriptional regulation as depicted in Fig. 1.

Paradoxical Effects of Fat on Hepatocytes and HSC

Our findings to date support that the “fat-storing” phenotype is good for HSC quiescence and anti-fibrogenesis. However, fat accumulation in hepatocytes causes fatty liver as exemplified by alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NFLD). In fact, if we analyze the expression of adipogenic transcription factors described above in fatty livers of ALD and NFLD animal models, they are upregulated (unpublished results). These results suggest that adipogenic transcriptional regulation may participate in the genesis of fatty liver. Interestingly, under these adipogenic conditions, HSC are still activated and collagen gene expression increased. This

leads to an intriguing question as to why HSC are resistant to adipogenic signals that make hepatocytes steatotic in these models. An answer to this question may provide novel insights into not only paradoxical significance of fat in hepatocytes and HSC but also differential sensitivity of these two cell types to adipogenic environments in pathologic conditions.

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