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Protein tyrosine phosphatase 1B inhibits adipocyte differentiation and mediates TNFα action in obesity☆

Dan-Dan Song [a,b,1,2], Yue Chen [a,1], Zhi-Yong Li [b], Yun-Feng Guan [b], Da-Jin Zou [a,*], Chao-Yu Miao [b,**]

[a] Department of Endocrinology, Changhai Hospital, Second Military Medical University, Shanghai, China
[b] Department of Pharmacology, Second Military Medical University, Shanghai, China

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ABSTRACT

Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of systemic glucose and insulin homeostasis; however, its exact role in adipocytes is poorly understood. This study was to elucidate the role of PTP1B in adipocyte differentiation and its implication in obesity. During differentiation of 3T3-L1 white preadipocytes, PTP1B decreased progressively with adipocyte maturation. Lentivirus-mediated PTP1B overexpression in preadipocytes delayed adipocyte differentiation, shown as lack of mature adipocytes, low level of lipid accumulation, and down-regulation of main markers (PPARγ2, SREBP-1c, FAS and LPL). In contrast, lentivirus-mediated PTP1B knockdown accelerated adipocyte differentiation, demonstrated as full of mature adipocytes, high level of lipid accumulation, and up-regulation of main markers. Dominant-negative inhibition on endogenous PTP1B by lentivirus-mediated overexpression of PTP1B double mutant in Tyr-46 and Asp-181 residues (LV-D/A-Y/F) also stimulated adipogenesis, more efficient than PTP1B knockdown. Diet-induced obesity mice exhibited an up-regulation of PTP1B and TNFα accompanied by a down-regulation of PPARγ2 in white adipose tissue. TNFα recombinant protein impeded PTP1B reduction and inhibited adipocyte differentiation in vitro; this inhibitory effect was prevented by LV-D/A-Y/F. Moreover, PTP1B inhibitor treatment improved adipogenesis and suppressed TNFα in adipose tissue of obese mice. All together, PTP1B negatively regulates adipocyte development and may mediate TNFα action to impair adipocyte differentiation in obesity. Our study provides novel evidence for the importance of PTP1B in obesity and for the potential application of PTP1B inhibitors.

1. Introduction

Protein tyrosine phosphatase 1B (PTP1B) is a ubiquitously expressed non-transmembrane protein tyrosine phosphatase. It is associated with numerous diseases, including cancer, metabolic and cardiovascular diseases, autoimmune and neurological disorders [1]. PTP1B becomes the target of intensive investigation because of its importance in glucose and insulin homeostasis. Global PTP1B knockout mice exhibit remarkably lower adiposity and are protected against diet-induced obesity [2,3]. Several studies have shown that the effect of PTP1B on obesity seems tissue-specific. Muscle-, liver- and adipose-specific PTP1B deletion do not contribute to the beneficial effect on body fat; only neuron-specific PTP1B null mice demonstrate the same phenotype as global knockout ones [4–7]. Furthermore, the glucose transport in isolated adipose tissue from total [2,3,8] and adipocyte-specific [9] PTP1B knockout mice have no difference compared with controls, suggesting an inessential role of PTP1B on insulin signaling in adipocytes. However, accumulating evidence shows that PTP1B is up-regulated in adipose tissue of obesity [9–14]. This promotes us to explore the possibility that PTP1B is involved in other important functions rather than insulin sensitivity in adipose.

As the essence of obesity, the imbalance between energy intake and expenditure leads to a pathologic accumulation of lipid. Adipose cells become hypertrophic cells in response to excess energy, which is accompanied with a dysregulation of PPARγ activity [15,16] and a failure of adipocyte differentiation [17–19]. Hence obese individuals are having difficulty in producing new fat cells, resulting in a lipid overload and the flow of fatty acids into circulation, muscle and liver [20]. During this progression, inflammatory response plays an important role through the actions of adipokines such as TNFα [21]. The expression of TNFα in adipose tissue is induced in a variety of obese rodents and humans [22–25], which impairs the differentiation

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* Correspondence to: D.-J. Zou, Department of Endocrinology, Changhai Hospital, Second Military Medical University, 168 Chang Hai Road, Shanghai 200433, China.
** Correspondence to: C.-Y. Miao, Department of Pharmacology, Second Military Medical University, 325 Guo He Road, Shanghai 200433, China. Tel.: +86 21 81871271; fax: +86 21 65493951.
E-mail addresses: zwjd22@medmail.com.cn (D.-J. Zou), cymiao@smmu.edu.cn (C.-Y. Miao).
[1] Dan-Dan Song and Yue Chen contributed equally to this article.
[2] Present address: Department of Endocrinology, Guangzhou General Hospital of Guangzhou Military Command Region, Guangzhou, China.

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Table 1
Primers used for real-time PCR.

Gene	5′-3′ sequence	
PTP1B	Forward	AGTACGACAGTTGGAGTTGG
	Reverse	TCGGGTGGAAGGTCTAGATC
β-actin	Forward	TGTCCACCTTCCAGCAGATGT
	Reverse	AGCTCAGTAACAGTCCGCCTAGA
Peroxisome proliferator-activated receptor γ2 (PPARγ2)	Forward	TCGCTGATGCACTGCCTATG
	Reverse	GAGAGGTCCACAGAGCTGATT
CCAAT/enhancer binding protein α (C/EBPα)	Forward	CAAGAACAGCAACGAGTACCG
	Reverse	GTCACTGGTCAACTCCAGCAC
Sterol regulatory element-binding proteins-1c (SREBP-1c)	Forward	GCAGCCACCATCTAGCCTG
	Reverse	CAGCAGTGAGTCTGCCTTGAT
Fatty acid synthetase (FAS)	Forward	GGAGGTGGTGATAGCCGGTAT
	Reverse	TGGGTAATCCATAGAGCCCAG
Lipoprotein lipase (LPL)	Forward	GGGAGTTTGGCTCCAGAGTTT
	Reverse	TGTGTCTTCAGGGGTCCTTAG
Tumor necrosis factor α (TNFα)	Forward	CCCTCACACTCAGATCATCTTCT
	Reverse	GCTACGACGTGGGCTACAG

of preadipocytes, abnormalizes the secretion of adipokines, promotes the infiltration of inflammatory cells, and finally contributes to an alteration of adipose microenvironment. Although it has been demonstrated that TNFα inhibits adipocyte differentiation in obesity [26–29], the underlying molecular mechanism has not been understood currently.

Adipocyte differentiation is under transcriptional control. So far, several studies have shown that PTP1B may be involved in the transcription of adipogenic markers. Culture of immortalized brown preadipocytes from PTP1B knockout mice shows a trend in up-regulation of adipogenic genes [30,31], but PTP1B antisense oligonucleotide treatment in ob/ob and db/db mice comes to an opposite result [32,33]. The ambiguity of these data may be derived from different experimental methods and subjects; nevertheless, it sheds light on the importance of PTP1B in adipogenesis. Here, we use lentivirus-mediated overexpression and knockdown of PTP1B as well as overexpression of PTP1B double mutant in white preadipocytes to study the role of PTP1B in adipocyte differentiation. Further, we explore whether PTP1B is involved in the inhibitory effect of TNFα on adipocyte differentiation in obesity.

2. Materials and methods

2.1. Lentivirus generation

PTP1B overexpression, RNA interference and mutant were prepared by Sunbio Medical Biotechnology (Shanghai, China). Briefly, mouse PTP1B cDNA, short hairpin RNA or mutant DNA was cloned as *Eco*RI fragment and recombined into lentiviral vector (Sunbio Medical Biotechnology, Shanghai, China) to produce lentiviral vector encoding PTP1B gene (LV-PTP1B), shRNA against PTP1B (sh-PTP1B), or PTP1B-D/A-Y/F double mutant (LV-D/A-Y/F), respectively. The constructs were verified by sequencing and then cotransfected with three packaging vectors (Addgene, MA, USA) into 293T cells. The supernatant was collected after 48 h and cleared of cell debris by filtering through a 0.45 μm filter. The titer of the lentivirus in the supernatant was determined by Real-time PCR according to the manufacturer's instructions. All the lentiviral vectors were fused with enhanced green

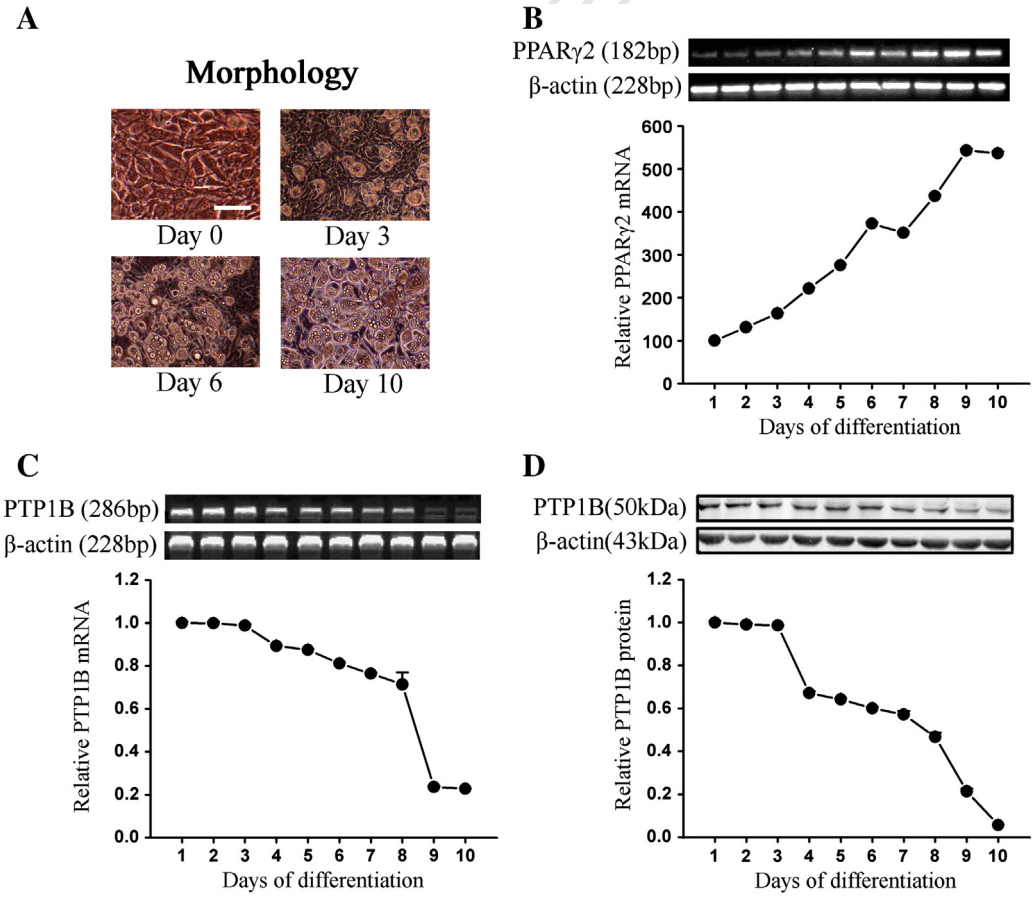

Fig. 1. PTP1B expression is negatively correlated with adipocyte differentiation. (A) Morphology of 3T3-L1 cells during differentiation. Preadipocytes become large, round and cytoplasm-abundant at day 3; lipid droplets appear at day 6; and more than 90% cells are differentiated into mature adipocytes at day 10. Images are captured using a 20× objective. Bar, 50 μm. (B–D) Time courses of PPARγ2 and PTP1B expression in 3T3-L1 cells during differentiation. Protein and mRNA levels are all expressed relative to preadipocytes (day 0) and normalized to the housekeeping gene β-actin. Data are means ± SEM of three independent experiments.

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