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Identification and characterization of a novel mouse peroxisome proliferator-activated receptor α-regulated and starvation-induced gene, *Ppsig*

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Abstract

The peroxisome proliferator-activated receptor alpha (PPARα) has been known to play a pivotal role in maintaining the energy balance during fasting; however, the battery of PPARα target genes involved in this metabolic response is still not fully characterized. Here, we report the identification and characterization of *Ppsig* (for PPARα-regulated and starvation-induced *g*ene) with unknown biological function from mouse liver. Multiple *Ppsig* cDNAs which differed in the 3′-untranslated regions were identified. The open reading frame of *Ppsi*g cDNA is 1830 bp which encodes a protein of 67.33 kDa. *Ppsig* contains 11 exons spanning at least 10 kb. Although the exact biological function of *Ppsig* is still not known, we found that *Ppsig* mRNA transcript was dramatically up-regulated during 72 h fasting and following treatment with a potent PPARα agonist, in a tissue-specific and PPARα-dependent manner. A functional peroxisome proliferator-response element was found in the intron 1 of *Ppsig*, thus confirming that *Ppsig* is a novel direct mouse PPARα target gene. This finding might help in elucidating the transcriptional regulatory mechanism of *Ppsig* in the cellular response to fasting.

Keywords: Fasting; mRNA differential display; PPARα; PPRE; *Ppsig*

1. Introduction

The peroxisome proliferator-activated receptor alpha (PPARα), a nuclear hormone receptor and a ligand-dependent transcriptional factor, has been known to play a key role in many biological processes such as lipid metabolism (Lee, Kim, Zhao, Cha, & Kim, 2004), amino acid metabolism (Kersten et al., 2001) and glycogen metabolism (Mandard et al., 2007). It is known that PPARα mediates its action via modulations of its target gene expressions. PPARα regulates its target genes by forming heterodimer with retinoid X receptor alpha (RXRα) and binds to a DNA consensus sequence named as the peroxisome proliferator-response element (PPRE) on its target genes (Desvergne & Wahli, 1999). The first PPRE was identified in the 5′-flanking of rat acyl-CoA

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oxidase gene (Tugwood et al., 1992). Since then many PPREs have been reported in the proximal promoters of a large number of PPARα target genes (Mandard, Muller, & Kersten, 2004). It is anticipated that more new PPARα target genes are awaited to be discovered.

As part of our continued efforts in identifying novel PPARα target genes in response to the metabolic perturbation during fasting, we have used the fluorescent differential display (FDD) to compare the liver mRNA profiles of wild-type (WT) and PPARα-null (KO) mice under both fed and fasted conditions, and to isolate PPARα-dependent and fasting-responsive genes (Lee, Tian, Lee, & Cheung, 2002). In the present paper, we describe the isolation, cloning and characterization of a novel PPARα target gene named *Ppsig* (for PPARα-regulated and starvation-induced gene) from mouse liver. The exact biological function of *Ppsig* is not known. But we observed that *Ppsig* mRNA transcript was dramatically induced in organs involved in lipid metabolism in the WT mice during 72 h fasting. No such induction was observed in the fasted KO mice, suggesting that PPARα is required for the transcriptional up-regulation of *Ppsig* during fasting. Indeed a functional PPRE was found in the intron 1 of *Ppsig*, thus confirming that *Ppsig* is a novel PPARα target gene which might play a significant role in the cellular response during energy deprivation.

2. Materials and methods

2.1. Animal and treatment

Male WT and KO mice were used (Lee et al., 1995). For the starvation experiment, fasted animals were deprived of food for 72 h prior to collection of tissue samples. For the Wy-14,643 [(4-chloro-6-(2,3-xylidino)-2-pyrimidinyl-thio)acetic acid] experiment, animals were provided with a 0.1% (w/w) Wy-14,643 (Chemsyn Laboratories, Lenexa, KS) rodent chow diet (Bioserv, Frenchtown, NJ) for 2 weeks ad libitum.

2.2. Fluorescent differential display

FDD was used to compare the mRNA expression profiles between the WT and KO mice under both fed and fasting states (Lee et al., 2002). The AP3 and ARP12 primers (Table 1) were used for the FDD analysis.

2.3. Rapid amplification of cDNA ends (RACE)

The full-length mouse liver *Ppsig* cDNA sequence was obtained by 5′- and 3′-RACE reactions with a GeneRacer™ cDNA amplification kit (Invitrogen)

Table 1
Primers used for FDD, 5′- and 3′-RACE, TSS, PPRE, Northern blot and RT-PCR analyses

Name	Sequence (5′–3′)	Application
AP3	ACGACTCACTATAGGGCTTTTTTTTTTTGG	FDD
ARP12	ACAATTTCACACAGGAGGTACTAAGG	
5′-RACE *Ppsig* reverse	GTAGGAGGGAGGACACCACGAGAA	5′-RACE
3′-RACE *Ppsig* forward	AATGCCTTCGTGGAAGCCTCTATGT	3′-RACE
Ppsig-RP154-IRD800	CAGCAGCAGAGCAGTGATCCACAT	TSS
Ppsig-FP(−2906)	ctaGCTAGCtagCGGGTGAAGAGGACAACTTT	pGL3-*Ppsig* (−2906/+145)
Ppsig-RP(+145)	ccgCTCGAGcggGGCGGTCGCTTGACATCC	
Ppsig-FP(+120)	cggGGTACCccgTTCGCCGAGGATGTCAAG	pGL3-*Ppsig* (+120/+461)
Ppsig-RP(+461)	ccgCTCGAGcggCCCGAGCATGCACAGAAG	
Mcd-FP(−311)	cggGGTACCccgAAGCGCGTGCGTAGTGG	pGL3-*Mcd*
Mcd-RP(+36)	ccgCTCGAGcggGCGCCGAGCCCTCAAGC	
Acbp-FP(–392)	cggGGTACCccgATGGTGTTCTCAGTTTCTG	pGL3-*Acbp*
Acbp-RP(+979)	ccgCTCGAGcggCCTAAGAGAGAGGGAGAG	
PPARα-FP423	cgcGGATCCgcgGCCACATCCATCCAACATG	pSG5-PPARα
PPARα-RP1849	cgcGGATCCgcgAAGATCAGTACATGTCTCTG	
RXRα-FP171	ggaAGATCTtccCGCAGACATGGACACCAA	pSG5-RXRα
RXRα-RP1752	ggaAGATCTtccGGCCAAAGGCAAGCATGA	
Ppsig-SEQ138	GAGCCCTGGCAGCAACAT	Northern blot and RT-PCR of *Ppsig*
Ppsig-SEQ1948R	GCCTTGACCTTTGAGCC	
Actb-FP2	CCCAGAGCAAGAGAGGTA	RT-PCR of *Actb*
Actb-RP2	CCGATCCACACAGAGTAC	
Gapdh-FP191	TCCACTCACGGCAAATTCAACG	RT-PCR of *Gapdh*
Gapdh-RP1017	TCCACCACCCTGTTGCTGTA	

The restriction sites are underlined and the nucleotides flanking the restriction sites are in lowercases.