Biochimie 96 (2014) 166–172

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Mini-review

Function of seipin: New insights from Bscl2/seipin knockout mouse models

Lucile Dollet [a], Jocelyne Magré [a], Bertrand Cariou [a,b,c], Xavier Prieur [a,c,*]

[a] INSERM UMR 1087, IRS-UN, 8 quai Moncousu, BP 70721, 44007 Nantes Cedex 1, France
[b] Department of Endocrinology, l'Institut du Thorax, Hôpital Nord Laennec, Boulevard Jacques-Monod, Saint-Herblain, 44093 Nantes Cedex 1, France
[c] University of Nantes, 2 Chemin de la Houssinière, 44300 Nantes, France

ARTICLE INFO

Article history:
Received 2 May 2013
Accepted 20 June 2013
Available online 2 July 2013

Keywords:
Bscl2/seipin
Lipodystrophy
Thiazolidinedione
Mouse embryonic fibroblasts
Adipocyte differentiation
Lipid droplet

ABSTRACT

Mutations in BSCL2/seipin cause Berardinelli-Seip congenital lipodystrophy (BSCL), a rare recessive disorder characterized by near absence of adipose tissue and severe insulin resistance. Since the discovery of the gene in 2001, several cellular studies intended to unravel the biological function of seipin and revealed that seipin-deficiency alters adipocyte differentiation and lipid droplet morphology. However, the exact function of the protein remains unclear and the pathophysiology of BSCL in patients carrying BSCL2/seipin mutations is poorly understood. A major breakthrough in the field of seipin came recently, with the demonstration by three independent groups that *Bscl2*-deficient mice ($Bscl2^{-/-}$) developed severe lipodystrophy with only residual white and brown fat pads, validating a critical role for seipin in adipose tissue homeostasis.

Using *in vivo*, *ex vivo* and *in vitro* methods, these studies demonstrate that seipin plays a key role in adipogenesis, lipid droplet homeostasis and cellular triglyceride lipolysis. In addition to adipose tissue impairment, $Bscl2^{-/-}$ mice are diabetic and display severe hepatic steatosis. Treatment with thiazolidinediones (TZD) in $Bscl2^{-/-}$ mice increases adipose tissue mass and partially rescues the metabolic complications associated with BSCL, highlighting that lipoatrophy is the major cause of the BSCL phenotype. Except an unexpected hypotriglyceridemia, $Bscl2^{-/-}$ mice phenotype represents an almost perfect picture of the human disease.

This review analyses how these studies using $Bscl2^{-/-}$ mice brought new insights into seipin function and the mechanisms involved in the pathophysiology of BSCL. We also analyse some of the human data in the light of the mouse phenotyping and discuss the validity of $Bscl2^{-/-}$ mice model to test pharmaceutical approaches for treating BSCL and its associated metabolic complications.

1. Introduction

Berardinelli-Seip congenital lipodystrophy (BSCL), also known as congenital generalized lipodystrophy, is a rare autosomal recessive disease, characterized by a near total absence of adipose tissue from birth [1]. BSCL is associated with muscular hypertrophy and organomegaly, such as hepatomegaly, splenomegaly and cardiac hypertrophy. Hypertriglyceridemia and liver steatosis also occur early in patients with BSCL, followed by insulin resistance that progressively worsens during childhood, ultimately leading to type 2 diabetes at adolescence [2]. Other common clinical manifestations include acanthosis nigricans and polycystic ovarian disease. Some cases of mild mental retardation were also described. Children present a voracious appetite, due to a deficiency in the satiety hormone leptin. Globally, BSCL patients have a reduced life expectancy, mainly due to an increased prevalence of pancreatitis, liver cirrhosis, heart failure and cardiac arrhythmias linked to cardiomyopathy [2–4].

In the majority of cases (95%), BSCL is caused by mutations occurring in the genes encoding either 1-acylglycerol-3-phosphate acyltransferase-β (*AGPAT2*/BSCL1) [5] or seipin (BSCL2) [6]. *AGPAT2* is highly expressed in adipose tissue and catalyses the formation of phosphatidic acid (PA), a key intermediate step in the synthesis of triglycerides (TG) and phospholipids [7]. A non-sense mutation in *CAV1*, encoding caveolin-1, has been identified in a Brazilian patient as a new cause of BSCL [8]. Caveolin-1 is a structural protein of caveolae involved in the uptake of free fatty acid (FFA) and their

* Corresponding author. INSERM, UMR 1087, IRS-UN, 8 quai Moncousu, BP 70721, 44007 Nantes Cedex 1, France. Tel.: +33 2 28 08 01 66.
E-mail address: xavier.prieur@univ-nantes.fr (X. Prieur).

0300-9084/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biochi.2013.06.022

conversion to TG in the lipid droplet (LD) [9,10]. A few patients presenting with BSCL and in some cases muscular dystrophy have mutations in *PTRF*, encoding polymerase I and transcript release factor (also named cavin-1) which is involved in the biogenesis of caveolae and the regulation of caveolin-1 and caveolin-3 expression [11,12]. In some patients with BSCL, the disease is not linked to either of these loci suggesting the implication of other BSCL-causative genes that remain to be identified.

The generation of mouse models of inherited lipodystrophy was a major breakthrough in the understanding of the pathogenesis of generalized lipodystrophy and its associated metabolic complications [13]. The A-ZIP [14] and the aP2-SREBP1c lipoatrophic mice [15] demonstrated the crucial contribution of the adipose tissue in glucose and lipid homeostasis. More specifically, regarding BSCL, the phenotyping of *Agpat2*-deficient (*Agpat2*$^{-/-}$) mice have led to a better understanding of the molecular mechanisms involved in the development of the metabolic complications in BSCL1: *Agpat2*$^{-/-}$ mice are severely lipodystrophic with a total lack of white (WAT) and brown (BAT) adipose tissue [16]. In addition, *caveolin-1*-deficient mice also develop a lipodystrophic phenotype with reduced WAT mass, hypertriglyceridemia and insulin resistance [17]. These findings have led to perform the molecular screening of *CAV1* in patients with unexplained lipodystrophy [8]. Therefore, the generation of mouse model highlights the mechanisms underlying human lipodystrophic phenotype and brings to mind candidate genes for inherited lipodystrophy.

In 2001, when *BSCL2* was identified as a BSCL-causative gene, the function of its encoded protein, seipin, was unknown [6]. Since then several cellular studies suggested that seipin is involved in adipocyte differentiation and LD homeostasis [18–21]. Recently, three models of *Bscl2*-deficient (*Bscl2*$^{-/-}$) mice have been generated. In this review, we will focus on the phenotyping of these mouse models of BSCL2 and discuss their usefulness for the understanding of both BSCL pathophysiology and seipin molecular function.

2. Seipin has a key role in adipose tissue homeostasis

2.1. Seipin-deficiency strongly impairs adipocyte homeostasis and leads to lipodystrophy

In the last two years, three independent models of *Bscl2*$^{-/-}$ mice have been published (Table 1). Interestingly, all the three models of *Bscl2*$^{-/-}$ mice display a severe and consistent lipodystrophy with a dramatic loss of fat mass as assessed by magnetic resonance imaging (MRI) [22,23] or by microscanner analysis [24]. In accordance with lipodystrophy, the circulating levels of the main adipokines, leptin and adiponectin, are strongly decreased. However, *Bscl2*$^{-/-}$ mice are not totally lipoatrophic, at least before the age of 6 months, since residual fat pads could be detected after careful dissection in some visceral and subcutaneous locations like inguinal, epididymal or mesenteric fat. Only the gonadal fat pad appears totally absent [22–24]. Histological analysis of WAT shows immature adipocytes with a defect in LD development [22,23]. In all *Bscl2*$^{-/-}$ models, BAT mass is also reduced, with a loss of the multilocular LD phenotype [23]. Thereby, the severe loss of adipose tissue observed in these mouse models is the first evidence that seipin-deficiency itself leads to severe lipodystrophy *in vivo*.

Table 1
Generation of *Bscl2*$^{-/-}$ mouse model.

	Gene mutation	Transcript	Predicted protein
Cui et al.	Deletion exon 3	No transcript	–
Chen et al.	Deletion exon 3	Remanent transcript	Generation of premature stop codon
Prieur et al.	Deletion exons 4–6	No transcript	Generation of a premature stop codon

WAT gene expression profile revealed a major alteration in the expression of key markers of the terminal adipocyte differentiation, such as adiponectin and the adipocyte fatty acid binding protein (aP2, also known as Fabp4) [24]. In another model, the expression of aP2 is not significantly altered, despite a tendency for a decrease [23]. Interestingly, the expression of brown adipocyte markers (such as the uncoupling protein 1 and Cidea) are markedly increased, pointing out browning of the residual white fat depots [23, Prieur et al., unpublished data]. In accordance with a functional browning, O_2 consumption is increased during adipocyte differentiation of *Bscl2*$^{-/-}$ mouse embryonic fibroblasts (MEFs) [23]. Finally, the mRNA levels of Pref1, a preadipocyte marker, is severely decreased in fat pads of *Bscl2*$^{-/-}$ mice [23]. Altogether, these results demonstrate that seipin-deficiency leads to severe lipodystrophy and strongly impairs the number and the property of mature adipocytes.

2.2. Seipin is required for full adipocyte differentiation

Several studies intended to unravel seipin function in the adipocyte using cell lines. A role for seipin in adipocyte differentiation is supported by the increase in seipin mRNA expression during hormone-induced adipogenesis in both human or mouse cells [18,19]. Furthermore, knock-down of seipin using short hairpin RNA in 3T3-L1 and C3H10T1/2 cell-lines leads to an impaired terminal adipocyte differentiation. The expression levels of C/EBPβ and C/EBPδ, two transcription factors involved in the early phase of adipogenesis, are not affected by seipin knock-down [18,19]. Consistently, the response to the bone morphogenetic protein 4 (BMP4), a key factor involved in the commitment in preadipocyte lineage, is not affected by seipin-deficiency [19]. In contrast, these reports established that seipin-deficiency strongly affects the markers of mature adipocytes with a decreased expression of C/EBPα, PPARγ and its target genes.

Studies using *Bscl2*$^{-/-}$ MEFs brought new insights into the adipocyte function of seipin. Both studies confirmed that seipin is essential for full adipogenesis [23,24]. Whereas Chen et al. confirmed that seipin-deficiency impacts mainly the late phase of adipogenesis [23], we described an additional defect earlier in adipogenesis, with a reduced C/EBPβ expression 8 h post-induction and a decrease in PPARγ and C/EBPα expression from day 2 of adipogenesis [24]. Both studies revealed that the impairment in adipogenesis gene expression pattern worsened during the time course of adipogenesis. Finally, PPARγ activation with thiazolidiniediones (TZDs) was able to partly rescue the adipogenesis default in the 3T3-L1 [19] and in the MEFs models [24]. However, TZDs failed to rescue adipogenesis in *Bscl2*$^{-/-}$ MEFs in another study [23]. The reasons for this discrepancy remain unclear since the protocol used for adipocyte differentiation seemed very similar. Altogether, these results suggest that seipin-deficiency does not prevent the commitment of the preadipocyte into adipocyte, but strongly affects the final maturation of the adipocyte. The mechanism leading to the impairment of adipogenesis has not been identified. Recently, Qiu et al. have shown that the over-expression of the A212P seipin mutant in 3T3-L1 cells induces ER stress and inflammatory response [25]. In this study, there is no knock-down or knockout cells for seipin, therefore it is difficult to state whether the cellular stress reported is specifically due to the over-expression of the A212P mutant or to the lack of functional seipin. Further studies with seipin-deficient cells are required to determine the relevance of ER stress induction as the cause of the adipogenesis defect in patients carrying a mutation in BSCL2.