Received: 5 January 2017 | Accepted: 6 January 2017

DOI 10.1002/jcp.25786

WILEY Journal of Cellular Physiology

MINI-REVIEW

Lkb1 regulation of skeletal muscle development, metabolism and muscle progenitor cell homeostasis

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Funding information
National Natural Science Foundation of China 31672427; Zhejiang Provincial Natural Science Foundation of China LR17C170001; "Hundred Talents Program" funding from Zhejiang University to TZS

Liver kinase B1 (Lkb1), also named as Serine/Threonine protein kinase 11 (STK11), is a serine/threonine kinase that plays crucial roles in various cellular processes including cell survival, cell division, cellular polarity, cell growth, cell differentiation, and cell metabolism. In metabolic tissues, Lkb1 regulates glucose homeostasis and energy metabolism through phosphorylating and activating the AMPK subfamily proteins. In skeletal muscle, Lkb1 affects muscle development and postnatal growth, lipid and fatty acid oxidation, glucose metabolism, and insulin sensitivity. Recently, the regulatory roles of Lkb1 in regulating division, self-renew, proliferation, and differentiation of skeletal muscle progenitor cells have been reported. In this review, we discuss the roles of Lkb1 in regulating skeletal muscle progenitor cell homeostasis and skeletal muscle development and metabolism.

KEYWORDS
Lkb1, muscle development, myoblast, STK11, satellite cell

1 | INTRODUCTION

Liver kinase B1 (Lkb1), also called Serine/Threonine protein kinase 11 (STK11), is a master kinase of the AMPK subfamily (Lizcano et al., 2004). Lkb1 phosphorylates and activates the AMPK subfamily kinases and plays crucial roles in regulating glucose homeostasis and energy metabolism in various metabolic tissues (Koh et al., 2006; Lizcano et al., 2004; Shan, Xiong et al., 2016; Shaw et al., 2005). In liver, deletion of Lkb1 leads to hyperglycemia and increases the expression of gluconeogenic and lipogenic related genes in adult mice (Shaw et al., 2005). In pancreas, Lkb1 deletion impacts the cytoskeletal organization and the pancreas endocrine compartment (Hezel et al., 2008). In adipose tissue, knockout of Lkb1 affects white and brown adipose tissues development and function (Shan, Xiong et al., 2016; Zhang, Wang, Song, & Zou, 2013). In skeletal muscle, loss of Lkb1 results in skeletal muscle dysfunction (Shan et al., 2014; Thomson et al., 2010), affects lipid and fatty acid oxidation (Jeppesen et al., 2013; Thomson et al., 2007), and increases glucose homeostasis and insulin sensitivity (Koh et al., 2006; Sakamoto et al., 2005).

Lkb1 also plays crucial roles in regulating various cellular processes including cell survival, cell division, cell proliferation, cell differentiation, and cell metabolism (Gan et al., 2010; Gurumurthy et al., 2010; Nakada, Saunders, & Morrison, 2010; Shan, Zhang, Xiong, Wang, & Kuang, 2016). Previous studies show that Lkb1 regulates quiescence, cell survival, cell cycle and energy metabolism in haematopoietic stem cells (Gan et al., 2010; Gurumurthy et al., 2010; Nakada et al., 2010). In adipocyte progenitor cells, Lkb1 deletion promotes differentiation and lipid accumulation of adipocyte progenitor cells (Gormand et al., 2014; Shan, Xiong et al., 2016). In adult beta cells, loss of Lkb1 increases cell size and cell proliferation (Fu et al., 2009). More recently, the roles of Lkb1 in regulating division, self-renew, proliferation, and differentiation of skeletal muscle progenitor cells have been reported (Mian et al., 2012; Shan et al., 2014; Shan, Zhang, Bi, & Kuang, 2015; Shan, Zhang et al., 2016). Thus, in this review, we mainly discuss the regulatory roles of

Lkb1 in regulating skeletal muscle development, metabolism and muscle progenitor cell homeostasis.

1.1 | Lkb1 regulation of skeletal muscle stem cell homeostasis

The roles of Lkb1 in skeletal muscle progenitor cells have been recently reported by different groups (Mian et al., 2012; Shan et al., 2014). Lkb1 is not only expressed in MyoD$^+$ muscle progenitors during embryonic development, but also expressed in adult quiescent satellite cells (Shan et al., 2014), indicating Lkb1 may play a role in muscle progenitor cells. To determine the role of Lkb1 in muscle progenitor cells, Shan et al. (2014) generated *MyoD-Lkb1* mice by crossing *MyoD-Cre* mice with *Lkb1$^{flox/flox}$* mice. In the *MyoD-Lkb1* mice, the expression of Lkb1 in muscle progenitor cells as well as in skeletal muscle tissues has been ablated. Firstly, deletion of Lkb1 activates quiescent satellite cells and promotes proliferation of muscle progenitor cells (Shan et al., 2014). Lkb1 ablation increases the number of satellite cells in resting muscles of adult *MyoD-Lkb1* mice (Shan et al., 2014). Moreover, in vivo and in vitro studies reveal that Lkb1 deficiency promotes proliferation of satellite cells as well as the satellite cell-derived primary myoblasts (Shan et al., 2014). At molecular level, Shan et al. (2014) demonstrate that deletion of Lkb1 promotes muscle progenitors proliferation through AMPK-mTOR dependent signaling pathway.

Secondly, Lkb1 deletion inhibits myogenic differentiation of skeletal muscle progenitor cells (Shan et al., 2014, 2015). Previous study reports that the expression and activity of Lkb1 are increased during myoblast differentiation (Mian et al., 2012). Before myogenic differentiation, the *MyoD-Lkb1* myoblasts express lower levels of differentiation gene MyoG, but higher levels of muscle progenitor marker gene Pax7, compared with the WT myoblasts (Shan et al., 2014). After myogenic differentiation, Lkb1 deficient muscle cells have lower differentiation and fusion index than the WT cells (Shan et al., 2014). Mechanistically, they report that Lkb1 deletion inhibits MyoG expression and myogenic differentiation via GSK3β signaling pathway (Shan et al., 2014). Consistently, Mian et al. (2012) also report that knockdown of Lkb1 impairs myoblast differentiation, whereas overexpression of Lkb1 accelerates myoblast differentiation. Taken together, these results suggest that Lkb1 positively controls myogenic differentiation of skeletal muscle progenitor cells.

Thirdly, loss of Lkb1 elevates ectopic lipid accumulation in muscle progenitor cells and myotubes (Shan et al., 2015). Before differentiation, Lkb1 deficient myoblasts have more lipid accumulation and higher levels of lipogenic related genes than the WT myoblasts (Shan et al., 2015). After adipogenic differentiation, Lkb1 deficient progenitor cells differentiated into adipocyte-like cells with lots of lipid droplets and higher expression of adipogenic related genes (Shan et al., 2015). These results, combine with the previous reports, suggest that Lkb1 deletion inhibits myogenic differentiation but promotes adipogenic differentiation of the Lkb1-deficient muscle progenitor cells (Mian et al., 2012; Shan et al., 2014, 2015).

Fourthly, Lkb1 deficiency affects the muscle progenitor cells fates and function (Shan et al., 2014; Shan, Zhang et al., 2016). Lkb1 deficiency dramatically upregulates Pax7 expression and affects muscle progenitor cell fate decision by promoting self-renew and proliferation division but inhibiting differentiation division (Shan, Zhang et al., 2016). Consistently, in myoblasts, knockout of Lkb1 increases the number of self-renewal (Pax7$^+$/MyoD$^-$) and proliferation (Pax7$^+$/MyoD$^+$) cells, but decreases the proportion of differentiation (Pax7$^-$/MyoD$^+$) cells (Shan et al., 2014). Moreover, the functions of muscle progenitor cells are affected by Lkb1 deletion, as evidenced by decreased muscle regeneration capacity in damaged muscle in vivo (Shan et al., 2014). Moreover, they report that deletion of Lkb1 regulates Pax7 expression and cell fates of muscle progenitor cells through activating Notch1 signaling pathway (Shan, Zhang et al., 2016). Indeed, it has been reported that Notch signaling pathway plays important roles in regulating Pax7 expression and muscle stem cell fate (Conboy and Rando, 2002; Wen et al., 2012). Taken together, these results establish a crucial role of Lkb1 in regulating muscle stem cell fate and function.

1.2 | Lkb1 regulation of skeletal muscle development and metabolism

Results from tissue-specific Lkb1 knockout mouse models demonstrate that Lkb1 functions as a critical regulator in tissue development and function (Shan, Xiong et al., 2016; Shaw et al., 2005; Shan et al., 2014; Thomson et al., 2010; Zhang et al., 2013). To investigate the role of Lkb1 in skeletal muscle, muscle-specific Lkb1 knockout mouse models have been generated by different groups using different *Cre* lines (Koh et al., 2006; Sakamoto, Goransson, Hardie, & Alessi, 2004; Shan et al., 2014; Thomson et al., 2010). Thomson et al. (2010) generated a muscle-specific Lkb1 deletion mouse model using *MCK-Cre*. They report that the old Lkb1-KO mice have myopathy phenotype, reduction of body weight and fast-twitch skeletal muscle weight, and dysfunction of hindlimb (Thomson et al., 2010), indicating Lkb1 plays an essential role in maintaining the function of skeletal muscle. Recently, Shan et al. (2014) generated *MyoD-Lkb1* mice and showed that *MyoD-Cre* mediated deletion of Lkb1 affects skeletal muscle development and growth. Their results show that *MyoD-Lkb1* mice have an obviously kyphosis and severe muscle dystrophy with high degree of centronuclear myofibers and dysfunction of hindlimb (Shan et al., 2014). The muscle mass and total fiber numbers of the tibialis anterior, extensor digitorum longus and soleus muscles are dramatically reduced in *Myod-Lkb1* mice compared to the WT mice (Shan et al., 2014). These results suggest that Lkb1 plays essential role in skeletal muscle development, postnatal muscle growth, and maintenance.

Lkb1 deletion in skeletal muscle regulates the glucose metabolism, insulin sensitivity, and lipid oxidation (Jeppesen et al., 2013; Koh et al., 2006; Thomson et al., 2007, 2010). Skeletal muscle-specific knockout of Lkb1 decreases fasting blood glucose and serum insulin concentrations, enhances insulin-stimulated muscle glucose uptake, and improves glucose homeostasis in adult mice (Koh et al., 2006). Sakamoto et al. (2005) show that the basal activity of the AMPKa2 isoform, as well as the phosphorylation of acetyl CoA carboxylase-2 (AMPK downstream target), was greatly reduced in Lkb1-lacking

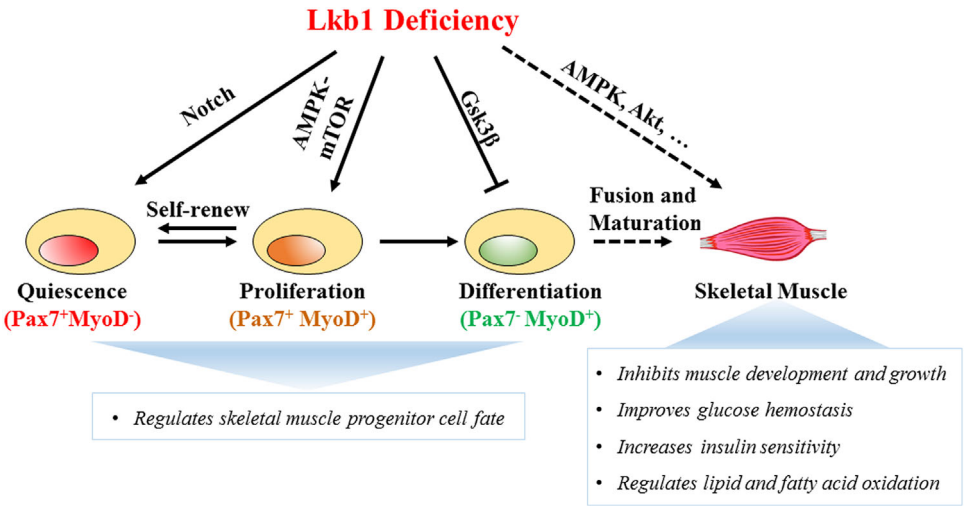

FIGURE 1 Lkb1 deletion affects skeletal muscle development, metabolism and muscle progenitor cell homeostasis. In skeletal muscle progenitor cells, deletion of Lkb1 promotes self-renewal and proliferation, but inhibits differentiation. In mature skeletal muscle, Lkb1 deletion results in defect of skeletal muscle development and postnatal growth, improves glucose homeostasis and insulin sensitivity, and affects lipid and fatty acid oxidation

muscle. In addition, the activation of AMPKa2 isoform by activator (such as AICAR, phenformin) treatments or exercise is dependent on Lkb1 (Sakamoto et al., 2005). Moreover, loss of Lkb1 inhibits the glucose uptake stimulated by AICAR or muscle contraction in skeletal muscle, indicating the importance of Lkb1 in regulating AMPK activity and cellular energy metabolism (Sakamoto et al., 2005). However, in rat skeletal muscle, Sakamoto et al. (2004) showed that the effects of exercise, phenformin, and AICAR on metabolic processes are mainly mediated through activation of AMPK rather than through LKB1 or the AMPK-related kinases (such as QIK, QSK, and MARK4). In addition, Lkb1 is an important regulator of fatty acid oxidation and malonyl-CoA levels in skeletal muscle (Thomson et al., 2007). Jeppesen et al. (2013) show that muscle-specific Lkb1 deletion mice exhibit decreased fatty acid (FA) oxidation and the expression of FA oxidation related genes during treadmill exercise, indicating Lkb1 is important for FA oxidation in muscle during exercise. In addition, Lkb1 regulates glucose and lipid metabolism in cultured muscle cells (Imai, Inukai, Ikegami, Awata, & Katayama, 2006). These results suggest that Lkb1 also plays critical role in regulating development and metabolism in skeletal muscle.

In summary, Lkb1 affects skeletal muscle development, metabolism and muscle progenitor cell homeostasis (Fig. 1). In skeletal muscle progenitor cells Lkb1 deficiency upregulates Pax7 expression, promotes proliferation and inhibits differentiation through Notch, mTOR, or GSK3β signaling pathways, respectively (Fig. 1). In skeletal muscle, loss of Lkb1 not only impairs muscle development and growth, but also affects glucose homeostasis and insulin sensitivity (Fig. 1). Thus, regulation of Lkb1 expression and activity may be a useful strategy for controlling metabolism and development of skeletal muscle in the future.

ACKNOWLEDGMENTS

The project was partially supported by the National Natural Science Foundation of China (31672427), the Zhejiang Provincial Natural Science Foundation of China (LR17C170001), and the "Hundred Talents Program" funding from Zhejiang University to TZS.

DISCLOSURES

The authors have no disclosures or other conflicts of interest to report.

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How to cite this article: Shan T, Xu Z, Liu J, Wu W, Wang Y. Lkb1 regulation of skeletal muscle development, metabolism and muscle progenitor cell homeostasis. *J Cell Physiol*. 2017;232:2653–2656. https://doi.org/10.1002/jcp.25786