Hepatic LKB1 and NAFLD

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Hepatic lipid droplets, expression of AR, and phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) increased in the presence of testosterone. Concurrently, the expression of LKB1, an upstream regulator of AMPK, was increased by testosterone treatment.

Keywords: testosterone ; LKB1 ; NAFLD ; AMPK

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to the state of steatosis, which is characterized by the excessive accumulation of triglycerides (TGs) in hepatocytes, regardless of alcohol assumption [1][2]. NAFLD is distinguished from non-alcoholic steatohepatitis (NASH) by the presence of inflammation. NASH, which is accompanied by inflammation, is a more advanced form than NAFLD and can progress to cirrhosis and liver cancer as hepatocarcinoma [3][4]. Thus, NAFLD must be managed to prevent progression to NASH or beyond. Obesity is a representative risk factor related to the incidence of NAFLD [5]. This is attributed to the promotion of hepatic de novo lipogenesis (DNL), when the excess energy intake, composed of carbohydrates and/or fat, is met [6]. Acetyl-CoA carboxylase (ACC) is the key enzyme of the rate-limiting step since it converts acetyl-CoA to malonyl-CoA, known as an initiator of lipogenesis. AMP-activated protein kinase (AMPK) is an upstream regulator that functions as a sensor of the energy status. AMPK senses the AMP/ATP ratio in the body and regulates the phosphorylation of ACC [7]. In other words, the liver stores excess energy in the form of TGs into hepatocytes via AMPK-ACC signaling.

Additionally, sex differences have been mentioned as another risk factor. Statistical studies have consistently revealed that the incidence of NAFLD is higher in males than in females [8]. In males, the incidence increases around 45 years of age. Meanwhile, the levels of androgens begin to decline naturally [9]. This suggests that the presence and/or activation of androgens may be associated with NAFLD development. Sex steroid androgens participate in the regulation of energy metabolic homeostases, such as obesity and glucose intolerance, and are associated with lipid metabolism [10][11]. Androgens induce an increase in the expression and/or phosphorylation of AMPK, leading to the inactivation of APMK-ACC signaling [12]. However, the exact mechanism by which androgens modulate AMPK-ACC signaling is still unclear. Therefore, we have revealed the detailed action of the mechanism between androgens and AMPK-ACC signaling and whether this action is linked to NAFLD development.

Next, it focused on liver kinase B1 (LKB1), which is an upstream regulator of AMPK [13]. LKB1, encoded by the gene, interacts with testosterone as well as AMPK [14]. In particular, the mRNA expression of *LKB1* is decreased in adipocytes, depending on the concentration of the testosterone, which stimulates AMPK phosphorylation [15]. Interestingly, unlike adipocytes, it is observed that the mRNA expression of *LKB1* increased in the presence of testosterone in hepatocytes. Concurrently, a decrease in AMPK-ACC signaling and lipid accumulation was observed. This evidence suggests that testosterone, one of the androgens, suppresses NAFLD development as it plays an important role in the induction of transcription and/or translation of *LKB1*.

2. Hepatic LKB1 Reduces the Progression of Non-Alcoholic Fatty Liver Disease via Genomic Androgen Receptor Signaling

The incidence of NAFLD has increased in modern society, with an increasing population of individuals with obesity [16]. This surge is more prevalent in males over 45 years of age than in females of the same age [8]. Considering the decline in the total androgen levels in males around the age [9], the relationship between androgens and NAFLD is an important topic, although it is established that NAFLD is strongly linked to obesity and type2 diabetes [17][18]. In line with this argument, the present study examined the effects of a fat-rich diet and showed that orchiectomized mice, deprived of endogenous androgens, are predisposed to liver steatosis compared to naive mice. This evidence suggests that the presence of androgens might protect against the development of NAFLD.

When mice were fed a fat-rich diet, body weight and hepatic TG accumulation concurrently increased than the mice fed with a normal diet. The increase of hepatic TG accumulation was prominent in orchiectomized mice, compared to naïve mice and the mice injected with testosterone after castration. Meanwhile, unlike the increased TG accumulation, body weight was lowest in the orchiectomized mice, among the mice fed with a fat-rich diet. It is considered that testosterone suppresses hepatic TG accumulation and increases the body weight due to an increase in fat-free mass [19]. Along with the degree of TG accumulation, orchiectomized mice were also more vulnerable to inflammation and fibrosis. This implies that androgens can prevent the development of NAFLD and progression from NAFLD to steatosis-induced NASH with fibrosis, under a fat-rich diet.

First, we observed the fluctuation of hepatic DNL, as a recent study showed that hepatic TG accumulation is induced via DNL in patients with NAFLD [20]. AMPK-ACC signaling plays a major role in hepatic DNL [21]. The phosphorylation of AMPK and ACC decreased in orchiectomized mice, leading to a reduction in hepatic TG accumulation. Likewise, we found that AMPK-ACC signaling was inactivated when testosterone was administered, particularly under the condition of fatty acid supplementation and *AR* overexpression in vitro assay. Concurrently, the mRNA levels of *AR* increased in vivo when androgens levels were sufficient. In addition, the mRNA levels of *AR* increased in testosterone-treated cells. The *AR* mRNA levels surged more sharply when cells were treated with bicalutamide, an AR antagonist. This increase is considered a compensatory response to the steep suppression of AR activation [22], as the AR antagonist and the media to eradicate androgens inside the cells were simultaneously used. Unlike mRNA expression, the antagonistic effect of bicalutamide affected protein expression. This suggests that testosterone which is one of the androgens, by cooperating with AR, modulates hepatic TG synthesis and accumulation via AMPK-ACC signaling.

To assess whether the complex bound with testosterone and AR (AR complex) is directly recruited to the promoter of the *AMPK* gene, we performed a ChIP assay using the SNU-423 cells treated with testosterone. However, the results of the ChIP assay did not show the recruitment of the AR complex to the promoter of the *AMPK* gene. This result suggests that testosterone does not directly modulate *AMPK* expression. Despite the evidence, it could not exclude the genomic response of testosterone since the increased AMPK phosphorylation paralleled activation of the AR.

A recent study revealed that LKB1, an upstream regulator of AMPK, interacts with sex steroids in a genomic manner in adipocytes [15]. Therefore, we investigated whether LKB1 cooperates with androgens in the same way in hepatocytes. The results showed that *LKB1* mRNA expression increased when androgens and/or testosterone were present at sufficient levels both in vivo and in vitro, and in turn, the protein expression increased in vitro. Along with the increased LKB1 expression, the phosphorylation of AMPK and ACC was also raised. This implies that LKB1 mediates the signaling between androgens and AMPK phosphorylation in hepatocytes. Likewise, we speculated whether androgens drive *LKB1* expression in a genomic manner and performed a ChIP assay to assess the expression of the *LKB1* gene. The results showed that the AR complex was recruited to the promoter of the *LKB1* gene. As in previous ChIP studies on AR, the AR element sequence motif at the AR binding site is presently presumed to be “ACATTTGT” in part of the *LKB1* gene promoter [23].

In summary, our study suggests that androgens, particularly testosterone, can prevent hepatic TG accumulation by suppressing hepatic DNL. Androgens bound to AR modulate *LKB1* transcription through direct recruitment to the promoter of the *LKB1* gene. In turn, increased LKB1 expression modulates the phosphorylation of AMPK-ACC signaling, leading to the suppression of hepatic TG synthesis and accumulation. Therefore, these findings are meaningful because androgens deficiency might be another risk factor of NAFLD in males, and the control of hormone homeostasis prevents NAFLD from progressing to steatosis-induced NASH, cirrhosis, and hepatocellular carcinoma.

3. Conclusions

This entry suggests that NAFLD development was accentuated in conditions where the supply of androgens is limited. When AMPK-ACC signaling is generally considered the mainstream in de novo lipogenesis, testosterone modulates the signaling. Intuitively, testosterone could lead to the reduction of hepatic TG contents, confirmed that LKB1 regulated AMPK-ACC signaling and that testosterone interacts directly with the LKB1 gene to evoke a direct AR binding. This entry has increased our understanding of how testosterone acts to regulate NAFLD development and its relevance to androgen-responsive LKB1 in the male liver.

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