

Characterization of selenium-binding protein 1 (SeBP1) using SeBP1/2-double knockout mice Selenium-binding protein

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Characterization of selenium-binding protein 1 (SeBP1) using *SeBP1/2*-double knockout mice

(Selenium-binding protein (SeBP)1/2 ダブル欠損マウスによる SeBP1 の特性評価)

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【Objectives】

Mouse selenium-binding protein 1 (SeBP1), a homolog of human SELENBP1, encodes a ~56 kDa protein that has been implicated in antioxidant responses and tumor suppression [1-5]. However, its physiological function remains largely undefined. Prior studies from our laboratory demonstrated that hepatic SeBP1 expression in rats is markedly induced by exposure to dioxin-like compounds, including polychlorinated biphenyls (PCBs) and 3-methylcholanthrene (3-MC), suggesting a role for SeBP1 in xenobiotic response pathways [6-7]. Whether similar regulatory mechanisms are conserved in human cells remains unknown. In mice, Selenium-binding protein 2 (SeBP2), a homolog sharing ~98% sequence identity with SeBP1, has been identified as an acetaminophen (APAP)-binding protein [8]. In *SeBP1*-deficient mice, no overt alterations in response to APAP or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been observed, implying potential functional compensation by SeBP2 [7]. Given the low expression of SeBP2 in renal tissue, previous findings suggest SeBP1 deficiency may influence lipid metabolism through modulation of peroxisome proliferator-activated receptor alpha (PPAR α) signaling [9]. Nonetheless, the hepatic role of SeBP1 under physiological and toxicological conditions remains unresolved. This study aims to define the biological function of SeBP1 in the liver by generating and characterizing SeBP1 and SeBP2 double-knockout (*SeBP1/2*-DK) mice. Through integrative molecular and phenotypic analyses, this work seeks to clarify the contribution of SeBP1 to hepatic metabolism and chemical stress responses.

【Methods】**1. Animal experiments**

In the experiment described in Chapter 1 and Chapter 2, 8-week-old WT, *SeBP1*-SK, and *SeBP1/2*-DK mice were fasted for 20 h and euthanized by CO₂ inhalation. Livers and serum were snap-frozen, and stored at -80°C.

In the experiment described in Chapter 4, 8-week-old WT and *SeBP1/2*-DK mice were fasted for 20 h and treated with APAP (300 mg/kg, i.p.). Saline was used as the vehicle control. Liver and serum samples were collected 6 h later. All animal experiments were performed in accordance with the guidelines of the Kyushu University Animal Care and Experimentation Committee.

2. Schematic representation of human cell treatment procedures

HepG2 cells were seeded in 6-well plates at a density of 2×10^5 cells/well and cultured for 24 h. For section 3 of Chapter 2, HepG2 cells were transfected with pcDNA3.1-SELENBP1 using 1 mg/mL PEI MAX and incubated for 48 h. Cells and culture media were then collected for qRT-PCR, western blotting, and biochemical analyses.

For sections 2 and 3 of Chapter 3, after 24 h of seeding, the medium was replaced with a fresh

culture medium containing the drug at the indicated concentration. Cells were incubated for 48 h before being collected for further analysis.

For section 4 of Chapter 3, after 24 h of seeding, HepG2 cells were transfected with pcDNA3.1-human estrogen receptor- α (hESR1) using 1 mg/mL PEI MAX and incubated for 24 h. The medium was then replaced with a fresh culture medium containing the drug at the indicated concentration. Cells were incubated for 48 h before being collected for further analysis.

【Results】

1. The absence of *SeBP1* and *SeBP2* did not compromise the animals' overall development and reproductive ability. Male knockout mice exhibited a marked decrease in liver weight (% of body weight), suggesting that *SeBP1* and *SeBP2* may play critical roles in maintaining hepatic homeostasis. However, H&E staining, serum ALT and AST levels, and IL-1 β level indicated that *SeBP1* and *SeBP2* deficiency did not lead to overt liver injury. In contrast, the alterations in serum triglyceride, LDL-C, and HDL-C levels imply that the loss of *SeBP1* and *SeBP2* disrupts lipid metabolism, highlighting their potential involvement in the regulation of liver lipid homeostasis.

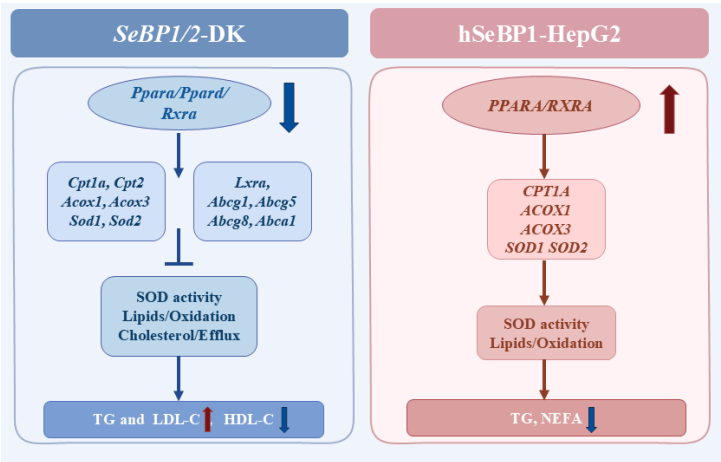


Fig.1 Role of *SeBP1* in Hepatic Lipid Metabolism

2. *SeBP1/2* promotes fatty acid oxidation in the liver via the PPAR α /CPT1A/ACOX1 pathway and affects cholesterol efflux via the PPAR γ /LXR α /ABCA1 pathway. *SeBP1/2* ablation may increase the risk of disrupted fatty acid oxidation and cholesterol efflux, highlighting their importance in lipid metabolism (Fig. 1).

3. Induction of SELENBP1 was not observed upon treatment with the aryl hydrocarbon receptor (AHR) agonists 3-MC and β -NF in HepG2 cells. In contrast, a clear induction of SELENBP1 was observed when hESR1 was overexpressed in HepG2 cells. Additionally, the AHR antagonist α -NF did not completely block 3-MC-induced SELENBP1 expression. These results suggest that SELENBP1 induction is driven by a cooperative interaction between the AHR and hESR1 pathways rather than solely relying on one receptor (Fig. 2).

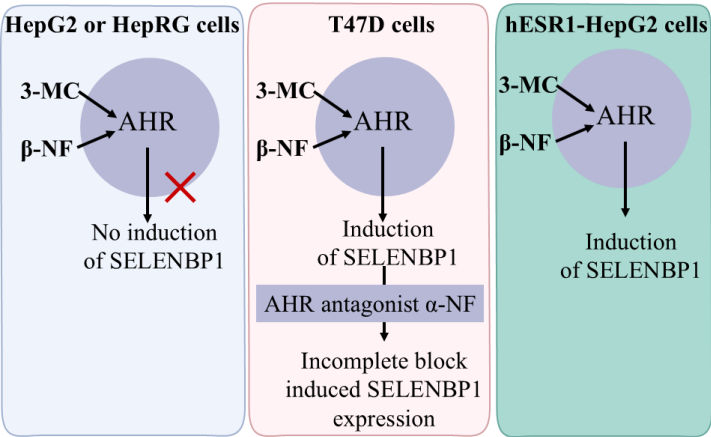


Fig. 2 SELENBP1 induction is driven by a cooperative interaction between the AHR and hESR1

- In *SeBP1*-SK mice, APAP induced SeBP2 protein expression in the liver. WT mice exhibited a reduction in body weight following APAP treatment, with a tendency toward a more pronounced effect trend in *SeBP1/2*-DK mice. Both groups showed elevated ALT and AST levels after APAP administration, with *SeBP1/2*-DK mice showing a more pronounced elevation, suggesting enhanced liver vulnerability. Additionally, increased serum IL-1 β levels confirmed the exacerbation of liver injury in *SeBP1/2*-DK mice. Importantly, *SeBP1/2* deficiency led to increased ERK1/2 protein levels and enhanced phosphorylation after APAP treatment. These results suggest that *SeBP1/2* protects the liver from APAP-induced injury (Fig. 3).

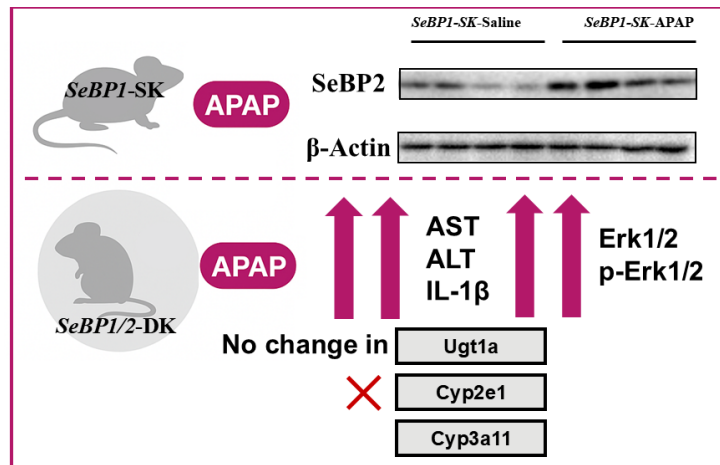


Fig.3 *SeBP1/2* protects APAP-induced liver injury by inhibiting Erk1/2 pathway

【Discussion】

The objectives of this study were to elucidate the physiological role of SeBP1 and its functional significance in AhR agonist-induced or APAP injury. To evaluate potential compensation by SeBP2, a *SeBP1/2*-double-knockout (*SeBP1/2*-DK) mouse model was generated. Although *SeBP1/2*-DK mice showed changes in organ weight, they maintained normal growth and survival, suggesting *SeBP1/2* is not essential for basic viability. The combined deletion of *SeBP1* and *SeBP2* reduced hepatic fatty acid oxidation and impaired biliary cholesterol excretion, indicating an unrecognized role in liver lipid metabolism. Given the high prevalence of lipid metabolic disorders, further research using high-fat diet models is needed to better understand the functions of *SeBP1* and *SeBP2* and their potential as therapeutic targets.

Patients with Yusho exhibit lipid metabolism abnormalities. To explore a potential link with SELENBP1 dysregulation, the effects of dioxin exposure on SELENBP1 expression were examined in human cells. Dioxin-like compounds did not significantly induce SELENBP1 in HepG2 cells but caused marked upregulation in T47D cells. This induction was only partially suppressed by an AHR antagonist, suggesting involvement of additional pathways. Since HepG2 cells lack endogenous ESR1, an ESR1 overexpression plasmid was introduced, and co-treatment with dioxin-like compounds significantly increased SELENBP1 expression. These findings indicate that SELENBP1 is regulated by AHR signaling and modulated by ESR1. This dual regulation provides insight into the interaction between xenobiotics and hormone signaling and may help clarify the mechanisms underlying lipid metabolism abnormalities in Yusho patients.

These findings demonstrate that hepatic loss of SeBP1 and SeBP2 exacerbates APAP-induced liver injury. This protective role does not appear to involve alterations in major drug-metabolizing enzymes, such as Cyp2e1, Cyp3a11, or Ugt1a isoforms, but is instead associated with enhanced inflammatory signaling. Notably, double knockout of *SeBP1* and *SeBP2* led to increased ERK expression and phosphorylation, which may contribute to the upregulation of pro-inflammatory cytokines, including IL-1 β , thereby amplifying hepatic injury. These results suggest that SeBP1 and

SeBP2 modulate inflammatory responses during APAP-induced hepatotoxicity and function as protective regulators of hepatic homeostasis under toxic stress.

In summary, this study provides new insights into the physiological functions of SeBP1 and SeBP2 and their involvement in both lipid metabolic dysregulation and APAP-induced liver injury, offering a novel perspective for understanding and potentially addressing liver-related pathologies.

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【Publication】

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