INVolVEMENT OF KLF4 AND HNF4α IN THE TRANSCRIPTIONAL REGULATION OF PCFT

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[Purpose] Proton-coupled folate transporter (PCFT), which has recently been identified as a transporter responsible for the intestinal absorption of folates and antifolate drugs, is known to be expressed in the uniquely limited region of upper and middle small intestine. The present study was conducted to elucidate the transcriptional regulation mechanism involved in such a unique expression profile and potentially in its alteration.

[Methods] Dual-luciferase reporter assays were performed in HEK293 cells with a series of reporter plasmids containing the 5'-flanking region of the human PCFT gene and several plasmids containing the cDNA of one of transcription factors that are known to be present in the small intestine.

[Results and Discussion] The luciferase activity derived from the reporter construct containing the 5'-flanking sequence of -1695/+96 was found to be enhanced extensively by a factor of about 16 by the introduction of Krüppel-like factor 4 (KLF4). Hepatocyte nuclear factor 4α (HNF4α) enhanced the luciferase activity modestly by a factor of about 2, but HNF1α, HNF4γ and several other nuclear factors did not. Interestingly, HNF4α was found to double the KLF4-induced luciferase activity synergistically. By assays using progressively deleted 5'-flanking sequences, it was suggested that the region of -147 to -95 is involved in the activation of PCFT promoter by KLF4. Finally, the binding of KLF4 to the region was demonstrated by the chromatin immunoprecipitation assay.

[Conclusions] The present study has revealed that KLF4 is a major nuclear factor involved in the activation of PCFT promoter. In addition, HNF4α was suggested to synergistically enhance the KLF4-induced activation. This mechanism would be at least in part involved in the transcriptional regulation of PCFT expression.

REGULATORY MECHANISM OF DRUGS-INDUCED BSEP INTERNALIZATION BY CONSTRUCTION OF SCREENING SYSTEM FOR BSEP INTERNALIZATION

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[Purpose] Canalicular secretion of bile acids mediated by the bile salt export pump (Bsep) constitutes the major driving force for generation of bile flow. Dysfunction of Bsep is known to cause cholestasis and reduce bile acids excretion, which finally leads to liver injury resulted from bile acids accumulation. Cyclosporine A (CsA), anabolic steroids, pill, etc, are known to induce cholestasis, but, their precise mechanisms which finally lead to liver injury are remained unclear. In the previous study, we clarified that the Bsep internalization was caused by the selective Ca²⁺-dependent protein kinase C (cPKC) activation. Therefore, we constructed the screening system for Bsep localization. And we examined the effect of cholestatic drugs on the canalicular localization of Bsep and the role of cPKC activation by using this screening system.

[Methods] McA-RH7777 cell derived from rat hepatoma stably expressing Bsep-GFP (green fluorescence protein) was treated with cholestatic compounds (CsA [10 μM], methyltestosterone (MT) [1 μM], chlorpromazine hydrochloride (CPZ) [10 μM], tauroliithocholate (TLC) [50 μM]). The Bsep-GFP localization was investigated by confocal laser scanning microscope. In addition, Go6976 (cPKC selective inhibitor) [1 μM] was treated simultaneously with the drugs.

[Results and Discussion] The canalicular internalization of Bsep was confirmed by the 20 ~30min after treatment of CsA, MT, CPZ and TLC. Moreover, these internalizations were partly suppressed by the treatment of Go6976. These results suggest that cholestasis with change of Bsep localization is caused by a lot of cholestatic drugs and commonly regulated by mechanism through the activation of cPKC. We are now investigating the precise mechanisms of Bsep internalization by using this screening system.