ROLES OF DNA METHYLATION AND METHYL-DNA BINDING PROTEINS IN THE EXPRESSION OF ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B3 IN HEPG2 CELLS

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[Purpose] Multispecific organic anion transporter, Organic Anion Transporting Polypeptide 1B3 (OATP1B3/SLCO1B3), is expressed in several cancer cell lines as well as in tumor tissues, apparently sensitizing the cells to some anti-cancer chemotherapeutics. Recently, we have demonstrated that CpG dinucleotides around the transcriptional start site (TSS) of OATP1B3 are differentially methylated among cancer cell lines, and the mRNA expression of OATP1B3 is stimulated by the treatment with DNA methylation inhibitor in OATP1B3-negative cell lines (Ichihara S, et al. Pharm Res. 2010). These results suggested that DNA methylation-dependent gene silencing is at least partly involved in the regulation of OATP1B3 expression. In the present study, we examined the roles of the methyl-DNA binding proteins (MeCP2, MBD1, and MBD2) in the DNA methylation-dependent gene silencing of OATP1B3 in HepG2 cells in which the lack of OATP1B3 expression has been attributed to the dense DNA methylation around the TSS of OATP1B3.

[Methods] mRNA expression of MeCP2, MBD1, and MBD2 was knocked down using siRNAs targeting each MBD. Knock-down efficiency and the mRNA expression of OATP1B3 were analyzed by quantitative RT-PCR.

[Results and Discussion] Expression of OATP1B3 mRNA was below the limit of detection in naïve HepG2 cells. Among the well-characterized MBDs, HepG2 cells express MeCP2, MBD1, and MBD2. Knock-down of MBD2 increased the mRNA expression of OATP1B3, whereas those of other MBDs had no effect. [Conclusions] These data suggest that MBD2 is involved in the epigenetic regulation of the OATP1B3 gene in HepG2 cells, together with DNA methylation.

CHARACTERIZATION OF THE NOVEL TISSUE-SPECIFIC PROMOTER OF THE SLC29A1 GENE

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[Purpose] Equilibrative nucleoside transporter 1 (ENT1, SLC29A1) is mainly expressed at the plasma membrane in various cells where it transports various antiviral or antitumor nucleoside analogs. Since the results of the recent studies suggest that ENT1 expression level is one of the determinants for efficacy of the nucleoside-based antitumor therapy, it is important to clarify the mechanisms by which SLC29A1 gene expression is regulated. Recently we have identified the novel promoter region located approximately 10 kbp upstream from the initially identified promoter region, termed P4 promoter. The aim of this study was to characterize the P4 promoter region of the SLC29A1 gene.

[Methods] The transcription start site (TSS) was determined by RNA ligase-mediated 5’ rapid amplification of cDNA ends. The promoter activity was determined by luciferase assay. mRNA expression profile was analyzed by reverse transcription-PCR. CpG methylation status of the promoter region was analyzed by bisulfate sequencing.

[Results and Discussion] ENT1 mRNA transcribed by P4 promoter, termed ENT1e1, showed the pancreas-specific expression profile among seven tissues analyzed. The results of deletion analysis showed that the minimal promoter activity was found within 240 bp from the TSS and that the GC-box and the CCAAT-box, which were located close to the TSS, played critical roles in the promoter activity. Accordingly, forced expression of Sp1 enhanced the promoter activity. The results of bisulfate sequencing showed that the CpG sequences in the GC-box were mostly unmethylated in the pancreatic genomic DNA, whereas they were highly methylated in the lung and kidney genomic DNA.

[Conclusion] The P4 promoter is activated through the GC- and CCAAT-boxes in a tissue-specific manner to generate ENT1e1 mRNA in the tissue. The tissue-specific epigenetic condition may be essential for the P4 promoter activation.