PGRMC1 MODULATES ACTIVITIES OF HUMAN DRUG-METABOLIZING CYTOCHROME P450S IN AN ISOFORM-DEPENDENT MANNER

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[Purpose] Progesterone receptor membrane component 1 (PGRMC1) is a member of the heme-1 domain family of proteins. PGRMC1 shares key structural motifs with cytochrome b5, but the function is not fully understood. It has been reported in an in vitro study that PGRMC1 binds to CYP21A2 and CYP51A1, enhancing their activities. In this study, we investigated the role of PGRMC1 on xenobiotic metabolism mediated by human cytochrome P450s.

[Methods] PGRMC1 expression levels in human liver microsomes were determined by Western blotting. PGRMC1 was co-expressed with CYP2C9, CYP2E1, or CYP3A4 in HepG2 cells using adenovirus expression system. The marker activities of each CYP, S-warfarin 7-hydroxylation, chlorzoxazone 6-hydroxylation, and testosterone 6β-hydroxylation, were measured to determine the effects of the co-expression of PGRMC1 on the enzymatic activities.

[Results and Discussion] PGRMC1 protein was highly expressed in human liver, showing slight interindividual variability (7-fold in 12 samples). The over-expression of PGRMC1 reduced the $V_{\text{max}}$ values of S-warfarin 7- and testosterone 6β-hydroxylation, but did not affect the $K_m$ values. In contrast, PGRMC1 did not alter chlorzoxazone 6-hydroxylation. Thus, it was demonstrated that PGRMC1 may have an impact on drug metabolism in a CYP isoform-dependent manner. We will report the results of co-immunoprecipitation analysis to demonstrate the direct interaction between the PGRMC1 and CYP2C9 and CYP3A4.

[Conclusions] We found that PGRMC1 modulates the activities of xenobiotic-metabolism by P450s in an isoform-dependent manner. Further studies are ongoing in our laboratory to determine whether it contributes to the interindividual variability in drug metabolism.

FUNCTIONAL EXPRESSIONS OF DRUG-METABOLIZING ENZYMES IN THE HUMAN HEPATOCELLULAR CARCINOMA CELL LINE CULTURED ON THE MICRO-SPACE CELL CULTURE PLATE

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[Purpose] Human hepatocellular carcinoma cell lines cultured in monolayer have been limited to use for drug metabolism study because of negligible activities of drug-metabolizing enzymes such as cytochrome P450s (CYPs). Recently 24-well plates arrayed with uniform micro-sized compartments on the bottom (micro-space cell culture plates) have been developed. In this study, we evaluated whether the human hepatocellular carcinoma cells cultured on the micro-space cell culture plates are useful for drug metabolism study.

[Methods] Human hepatocellular carcinoma FLC4 cells were cultured on the micro-space cell culture plates. Expression levels of various drug-metabolizing enzymes mRNAs were determined by DNA microarray analyses or real-time PCR analyses. Cells were treated with diclofenac (CYP2C9 substrate) or triazolam (CYP3A substrate), and their metabolites in the culture medium were determined by HPLC-UV methods.

[Results and Discussion] Spheroid cells formed on the micro-space cell culture plates showed higher expression levels of various drug-metabolizing enzymes and nuclear receptors than monolayer cells. Significant activities of CYP2C9 and CYP3A were detected in spheroid cells but not in monolayer cells. In only spheroid cells, expression levels of CYP3A4 and CYP2B6 mRNAs were induced by rifampicin (PXR ligand) and CITCO (CAR ligand), respectively.

[Conclusions] These results suggest that the micro-space cell culture plates are useful tools for drug metabolism study using the human hepatocellular carcinoma cell line.