Generation of isoform-specific monoclonal antibodies against human UDP-glucuronosyltransferases (UGTs)and tissue-specific expression of UGTs by epigenetic regulation

Dissertation abstract

Generation of isoform-specific monoclonal antibodies against human UDP-glucuronosyltransferases (UGTs) and tissue-specific expression of UGTs by epigenetic regulation

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Abstract

UDP-Glucuronosyltransferases (UGTs) are a family of membrane-bound enzymes that catalyze glucuronidation of endogenous and exogenous compounds. There are 19 different human UGT isoforms. The expression profiles of UGTs in human tissues at mRNA level had been studied. However, information regarding their protein levels was limited because of the lack of isoform-specific antibodies. In this thesis, first, peptide-specific monoclonal antibodies against human UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B10 were successfully prepared and were used to investigate the UGTs expression at protein levels. It was found the tissue distribution of UGT proteins was largely consistent with previous reports of mRNA expression. These antibodies would serve as a useful tool for further studies of UGTs to evaluate its physiological and pharmacological roles. Second, the underlying mechanism of the tissue-specific expression of UGT1A1 and UGT1A10 was investigated. UGT1A1 is predominantly expressed in the liver and intestine, but not in the kidney. Meanwhile UGT1A10 is exclusively expressed in the gastrointestinal tract but not in liver. It was suggested that DNA hypermethylation along with histone H3 hypoacetylation of the promoter of *UGT1A1* interfere with the binding of hepatocyte nuclear factor (HNF) 1α, resulting in the defective expression of UGT1A1 in the kidney. As for UGT1A10, DNA hypermethylation and H3K27 trimethylation would interfere with binding of HNF1α and caudal type homeobox 2, resulting in the defective expression of UGT1A10 in the human liver. This study could provide valuable information on the tissue distribution, interindividual variability, and tissue-specific regulatory mechanism of UGTs.

Dissertation abstract

UDP-Glucuronosyltransferases (UGTs) are a family of phase II drug-metabolizing enzymes that play key roles in the metabolism of endogenous and exogenous compounds. They catalyze the formation of glucuronides by the transfer of glucuronic acid from a cofactor uridine 5'-diphosphoglucuronic acid to hydroxyl, carboxyl, or amine groups of endogenous and exogenous substrates. Human UGTs are classified into two subfamilies, UGT1 and UGT2, based on similarities between their amino acid sequences and gene organization. To date, 19 human UGT isoforms have been identified. The expression profiles of UGTs in human tissues at mRNA level had been studied. However, information regarding their protein levels was limited because of the lack of isoform-specific antibodies, since UGTs share a high degree of amino acid similarities. In this study, first, peptide-specific monoclonal antibodies against

human UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B10 were prepared and were used to investigate the UGTs expression at protein levels. Second, the underlying mechanism of the tissue-specific expression of UGT1A1 and UGT1A10 were investigated focusing on epigenetic mechanism.

Generation of specific monoclonal antibodies against human UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B10 and evaluation of their protein levels in human tissues

Determination of the protein levels of the individual UGT isoforms in human tissues is required for the successful extrapolation of *in vitro* metabolic data to *in vivo* clearance. Most previous studies evaluating UGT isoform expression were limited to the mRNA level because of the high degree of amino acid sequence homology between UGT isoforms that has hampered the availability of isoform-specific antibodies. In this study, peptide-specific monoclonal antibodies against human UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B10 were successfully prepared. Using these antibodies, it was demonstrated that UGT1A6 and UGT1A9 proteins were expressed in both the kidney and the liver, but not in the small intestine, UGT2B4 and UGT2B10 were expressed only in the liver, UGT1A10 was expressed only in the small intestine, that are consistent with previous reports of mRNA expression. In a panel of 20 individual human livers, the UGT1A6, UGT1A9, UGT2B4, and UGT2B10 protein levels exhibited 10-, 9-, 6-, and 7-fold variability, respectively. Interestingly, their relative protein levels were not correlated with the corresponding mRNA levels, suggesting the importance of evaluating UGT isoform expression at protein levels. Thus, specific monoclonal antibodies against UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B10 were successfully generated and the distribution and relative expression levels of their protein in human tissues were evaluated. These antibodies would serve as a useful tool for further studies of UGTs to evaluate its physiological, pharmacological, and toxicological roles in human tissues.

Epigenetic regulation is a crucial factor in the repression of UGT1A1 expression in the human kidney

UGT1A1 catalyzes the metabolism of numerous clinically and pharmacologically important compounds such as bilirubin and SN-38. UGT1A1 is predominantly expressed in the liver and intestine, but not in the kidney. The purpose of this study was to uncover the mechanism of the tissue-specific expression of UGT1A1, focusing on its epigenetic regulation. Bisulfite sequence analysis revealed that the CpG-rich region near the *UGT1A1* promoter (-85

to +40) was hypermethylated (83%) in the kidney, whereas it was hypomethylated (24%) in the hepatocytes. A chromatin immunoprecipitation (ChIP) assay demonstrated that histone H3 near the promoter was hypoacetylated in the kidney but was hyperacetylated in the liver; this hyperacetylation was accompanied by the recruitment of hepatocyte nuclear factor (HNF) 1α to the promoter. The *UGT1A1* promoter in human kidney-derived HK-2 cells not expressing UGT1A1 was fully methylated, but was relatively unmethylated in human liver-derived HuH-7 cells expressing UGT1A1. Treatment with 5-aza-2'-deoxycytidine (5-Aza-dC), an inhibitor of DNA methylation, resulted in an increase of UGT1A1 mRNA expression in both cell types, but the increase was much larger in HK-2 cells than in HuH-7 cells. Overexpression of HNF1α in HK-2 cells resulted in an increase of UGT1A1 mRNA only in the presence of 5-Aza-dC. Thus, it was suggested that DNA hypermethylation along with histone hypoacetylation interferes with the binding of HNF1α, resulting in the defective expression of UGT1A1 in the human kidney.

Tissue-specific expression of human UGT1A10 by epigenetic regulation

UGT1A10 is exclusively expressed in the small intestine and colon, where it contributes to presystemic first-pass metabolism. Previous studies demonstrated that HNF1α and Sp1 as well as intestine-specific transcription factor caudal type homeobox (Cdx) 2 are involved in the constitutive expression of UGT1A10. However, HNF1α and Sp1 cannot solely account for the intestine-specific expression of UGT1A10, because the expression of these transcription factors are not confined to intestines, but rather abundantly expressed in the liver, where UGT1A10 is absent. The purpose of this study was to elucidate the underlying mechanism of tissue-specific expression of UGT1A10. Bisulfite sequence analysis revealed that the CpG-rich region around the *UGT1A10* promoter (-264 to +117) was hypermethylated (89%) in the epithelium of small intestine, whereas it was hypomethylated (6%) in the hepatocytes. Methylation of the *UGT1A10* promoter by *Sss*I methylase leads to an almost complete loss of transcriptional activity even under the overexpression of Cdx2 and HNF1α. The *UGT1A10* promoter in human liver-derived HuH-7 cells not expressing UGT1A10 was more highly methylated than in human colon-derived LS180 cells expressing UGT1A10. Treatment with 5-Aza-dC resulted in a much higher increase of UGT1A10 mRNA expression only in HuH-7 cells. Overexpression of HNF1α and Cdx2 resulted in an increase of UGT1A10 mRNA only in the presence of 5-Aza-dC in HuH-7 cells. A ChIP assay demonstrated that H3K27 around the promoter was trimethylated in the liver but not in the small intestine. Thus, DNA hypermethylation and H3K27 trimethylation would interfere with binding of HNF1α and Cdx2, resulting in the defective expression of UGT1A10 in the human

liver. Epigenetic regulation is a crucial determinant of tissue-specific expression of UGT1A10.

In summary, this study succeeded to prepare peptide-specific monoclonal antibodies against human UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B4, and UGT2B10 and could provide valuable information on the tissue distribution and interindividual variability of UGTs. In addition, it was found that the tissue-specific expression of UGTs is regulated by epigenetic mechanism including DNA methylation and histone modifications. Aging, sex, disease, and habits are known to affect epigenetic status. It would be of interest to investigate whether such factors affect drug response thorough the modulation of epigenetics of UGT.

References:

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学位論文審査結果の要旨

UDP-グルクロン酸転移酵素（UGT）は外因性および内因性化合物にグルクロン酸を付加し、体外への排泄を促進する酵素であり、ヒトでは19種類の分子種が存在する。本研究では、第一に、UGTタンパク質の定量的および定性的評価を可能にするため、医薬品代謝に重要な分子種であるUGT1A6, 1A8, 1A9, 1A10, 2B4, 2B10に対するモノクローナル抗体の作成に取り組み、特異的抗体の作成に成功した。これらの抗体を用いたウェスタンブロットにより、肝、腎、小腸において各UGTが組織特異的な発現分布を示すこと、肝臓における各UGT発現量の個人差が6-10倍あること、単位UGT当たりの酵素活性がUGT発現系タンパク質ではヒト組織中UGTタンパク質と比べて有意に低値を示すことを明らかにした。UGT発現系のデータからヒトにおけるクリアランスを外挿する際に注意すべき問題点を提起した価値ある研究成果である。第二に、肝に発現し腎に発現しないUGT1A1および小腸に発現し肝に発現しないUGT1A10の組織特異的発現機構について解析した。両者ともプロモーター領域のDNAが高度にメチル化されていることが転写因子の結合を妨げ、発現しない原因となっていることを明らかにした。すなわち、エピジェネティクスがUGTの組織特異的発現を制御する重要な因子であることを見出した。以上、本研究成果はヒトUGTの発現について新たな知見を与え、薬物動態の理解促進につながる価値あるものであり、博士（薬学）に値すると判定した。