Regulation of UDP-glucuronosyltransferase 1A genes in the intestine

SUMMARY

UDP-glucuronosyltransferases are a superfamily of enzymes involved in Phase II metabolism of small lipophilic chemicals; by conjugating these chemicals with sugars, UGTs render them more water soluble and readily eliminated. Human intestine is constantly exposed to lipophilic chemicals ingested as part of the diet, or as supplements or drugs. These chemicals can have positive bioactive effects or can potentially be toxic or carcinogenic. The activity of intestinal UGT enzymes is crucial as the first line of defence rendering these substances more water soluble, thus, facilitating their inactivation and excretion. The constitutive expression of different *UGT* genes is regulated in a defined tissue-specific manner. In addition, their expression within specific tissues can be induced by small molecules that include UGT substrates, allowing a feedback response. A cluster of *UGT* genes, *UGT1A8, -1A9, and -1A10,* are highly expressed in the intestine. However; while *UGT1A8* and *UGT1A10* are exclusively extrahepatic, *UGT1A9* expressed in both liver and intestine. With the evidence linking the risk of colorectal cancer with the level of intestinal UGT activity, it is of particular importance to determine how the spatiotemporal regulation of intestinal UGTs is mediated, both in terms of constitutive and inducible expression. CDX2 is an intestinal master transcription factor which works in partnership with HNF4α to control gene expression during intestinal development and intestinal epithelial renewal. In this study, we provided a defined novel mechanism by which CDX2 and HNF4α control *UGT1A8*, *-1A9* and *-1A10* expression at the promoter level. Using a variety of molecular techniques, we showed that CDX2 and HNF4α synergistically induce *UGT1A8-1A10* intestinal expression via a conserved composite element of 12 nt located at the proximal promoter of the three genes. Moreover, this work identified the first known functional CDX2 binding motif in *UGT1A9* helping to explain its intestinal expression. We also examined how HNF4α controls hepatic expression of *UGT1A9* leading to a model in which HNF4α acts via separate intestinal and hepatic regulatory modules in this gene. Overall our study showed that the CDX2 /HNF4α nexus that is critical in developmental patterning and maintenance of intestine also defines *UGT1A8, -1A9* and *-1A10* expression.

Inducible regulation of UGTs in the intestine involves dietary constituents and products of microbiota activity; reports show that the activation of UGTs by chemicals that are also UGT substrates constitutes a feedback-regulatory mechanism. Using Caco-2 cells carrying an integrated *UGT1A8* promoter-reporter construct, we screened for chemicals that could induce promoter activity. We identified genistein as a flavonoid that most potently induced the *UGT1A8* promoter. The effect of genistein on *UGT1A8* (as well as *-1A9 and -1A10*) expression was enhanced synergistically by butyrate. Butyrate is a fermentation product of gut microbiota and is well known as HDAC inhibitor. Via inducing chromatin remodelling, butyrate can promote access of ligand-induced transcription factors to target genes. We assessed whether genistein might function as ligand for various ligand-dependent transcription factors including PPAR. Our findings using antagonist assays supported involvement of PPARγ at the promoter and mRNA level. It is also possible that PPARγ activity is regulated post-transcriptionally via genistein; moreover, we found evidence that genistein can alter chromatin accessibility at the *UGT1A8* promoter. These studies define at least one pathway for inducible regulation of intestinal UGTs by flavonoids and butyrate; given that UGT1A8 conjugates many carcinogenic compounds, this induction may be involved in the protective effects of flavonoid and fibre rich diets on cancer risk.

To allow a better understanding of physiological aspects of constitutive and ligand-activated regulation of intestinal *UGT* genes, we established an intestinal organoid culture system using *UGT1A8* promoter-reporter transgenic mice. This model more closely represents the heterogeneity and structural features of normal intestine than cell line models but maintains benefits of cell lines such as amenability to genetic manipulation. In Matrigel based culture containing appropriate growth factors, spontaneous growth of enteroids or organoids from isolated intestinal crypts were observed, and these could be maintained throughout several passages as well as frozen for long-term storage. Although requiring further optimization, we found that genetic manipulation in organoids is possible using DNA and RNA transfection. We also showed that expression of *UGT1A8* and its main developmental and inducible regulators (CDX2, HNF4α, PPAR) in intestinal organoids is comparable to that in intact adult intestine. This preliminary exploration provides new scope for studies using humanized mice and organoid technology.