Significance of *UGT1A6, UGT1A9,* and *UGT2B7* genetic variants and their mRNA expression in the clinical outcome of renal cell carcinoma

Jun Matsumoto[1,*], Anzu Nishimoto[1], Shogo Watari[2], Hideo Ueki[3], Shoya Shiromizu[4], Naohiro Iwata[4], Tatsuaki Takeda[4], Soichiro Ushio[4], Makoto Kajizono[4], Masachika Fujiyoshi[5], Toshihiro Koyama[6], Motoo Araki[3], Koichiro Wada[7], Yoshito Zamami[4], Yasutomo Nasu[3], and Noritaka Ariyoshi[1]

[1] Department of Personalized Medicine and Preventive Healthcare Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata, Kita-ku, Okayama 700-8558, Japan

[2] Department of Urology, National Hospital Organization Okayama Medical Center, 1711-1 Tamasu, Kita-ku, Okayama 701-1192, Japan

[3] Department of Urology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata, Kita-ku, Okayama 700-8558, Japan

[4] Department of Pharmacy, Okayama University Hospital, 2-5-1 Shikata, Kita-ku, Okayama 700-8558, Japan

[5] Department of Pharmacy, Tottori University Hospital, 36-1 Nishi, Yonago, Tottori 683-8504, Japan

[6] Department of Pharmaceuticals Biomedicine, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata, Kita-ku, Okayama 700-8558, Japan

[7] Department of Urology, Faculty of Medicine, Shimane University, 89-1 Enya, Izumo, Shimane 693-8501, Japan

*Corresponding author:

Jun Matsumoto, Ph.D.

Address: 2-5-1 Shikata-cho, Kita-ku, Okayama, 700-8558, Japan

Telephone and fax number: +81-86-235-7873

Email: matsumotoj@okayama-u.ac.jp

ORCID iD: 0000-0001-7224-108X

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Abstract

UDP-glucuronosyltransferase (UGT) metabolizes a number of endogenous and exogenous substrates. Renal cells express high amounts of UGT; however, the significance of UGT in patients with renal cell carcinoma (RCC) remains unknown. In this study, we profile the mRNA expression of UGT subtypes (UGT1A6, UGT1A9, and UGT2B7) and their genetic variants in the kidney tissue of 125 Japanese patients with RCC (Okayama University Hospital, Japan). In addition, we elucidate the association between the *UGT* variants and UGT mRNA expression levels and clinical outcomes in these patients. The three representative genetic variants, namely, *UGT1A6* 541A>G, *UGT1A9* i399C>T, and *UGT2B7*-161C>T, were genotyped, and their mRNA expression levels in each tissue were determined. We found that the mRNA expression of the three UGTs (UGT1A6, UGT1A9, and UGT2B7) are significantly downregulated in RCC tissues. Moreover, in patients with RCC, the *UGT2B7*-161C>T variant and high UGT2B7 mRNA expression are significantly correlated with preferable cancer-specific survival (CSS) and overall survival (OS), respectively. As such, the *UGT2B7*-161C>T variant and UGT2B7 mRNA expression level were identified as significant independent prognostic factors of CSS and CSS/OS, respectively. Taken together, these findings indicate that UGT2B7 has a role in RCC progression and may, therefore, represent a potential prognostic biomarker for patients with RCC.

Keywords: genetic variant; polymorphism; renal cell carcinoma; survival; UDP-glucuronosyltransferase

Introduction

Renal cell carcinoma (RCC) is the most frequently observed cancer in the kidney and accounts for approximately 2% of all new cancer cases each year [1], with its incidence gradually increasing worldwide [2]. The clinical outcome for patients with RCC has improved with recent advances in cancer therapy; however, the overall prognosis remains unsatisfactory. Thus, the development of improved prevention and treatment strategies against RCC remains an important research target.

UDP-glucuronosyltransferase (UGT) is a superfamily responsible for catalyzing the conjugation of endogenous and exogenous substrates with glucuronic acid. There are 22 different human UGTs classified into four families based on sequence homology [3], with many compounds serving as UGT substrates. To date, cancer research on the role of UGT has focused on the metabolism of exogenous substrates, such as anticancer agents. Several anticancer agents, including irinotecan (SN-38), sorafenib, and tamoxifen, are metabolized by UGT leading to changes in the incidence of adverse effects and efficacy of the drugs [4, 5]. Moreover, UGT regulates the circulating levels of endogenous substrates, including steroids, bile acid, and eicosanoid [6]. Recent evidence has also shown significant correlations, which are independent of anticancer agent metabolism, between the expression levels of UGT and clinical outcomes in several cancer types [7-9]. In addition, *UGT* genes are highly polymorphic, leading to individual variations in their expression and enzymatic activities [10]. In fact, several *UGT* variants are considerably correlated with the metabolism pharmacokinetics of anticancer agents and clinical outcomes in cancer patients [10-13]. Thus, UGT has a role in cancer progression and has been proposed as a potential marker for cancer prevention and treatment. However, the role of UGT in cancer cells differs depending

on the type of cancer and individual UGT subtypes.

In humans, UGT is primarily expressed in the liver; nonetheless, individual subtypes of UGT exhibit different expression patterns and tissue distribution [10, 14]. The kidney expresses various metabolic enzymes, including UGT at high levels, indicating that renal cells possess notable metabolizing capacity [10, 15, 16]. Moreover, targeted mass-spectrometric quantifications show that the main UGT subtypes expressed in the kidney are UGT1A6, UGT1A9, and UGT2B7 [16]. Although knowledge regarding the role of renal UGT in the metabolism of endogenous and exogenous substrates is limited compared with that of hepatic UGT, it is expected that renal UGT contributes to the maintenance of homeostasis and metabolic drug clearance in the kidney. However, the clinical impact of genetic variants and expression of UGT on the outcome of patients with RCC remains unknown despite the fact that renal cells express high amounts of UGT. Therefore, further investigation into the role of UGT in RCC may contribute to a better understanding of the underlying malignant behavior of RCC.

Notably, the expression levels of UGT1A6, UGT1A9, and UGT2B7 proteins are considerably correlated with the associated mRNA levels in RCC tissues, indicating that the expression levels of these three UGT proteins are transcriptionally regulated in RCC [16]. Accordingly, in the present study, we aimed to profile the renal mRNA expression of UGT1A6, UGT1A9, and UGT2B7 and their representative genetic variants, while determining whether the mRNA expression of these UGTs (including the variants) are correlated with clinical outcomes in patients with RCC. To the best of our knowledge, this is the first study to provide novel insights into the clinical relevance of *UGT* variants and their mRNA expression in patients with RCC.

Materials and methods

Patients and tissue samples

A total of 125 Japanese patients with RCC who underwent surgery between March 2003 and December 2015 at the Okayama University Hospital (Okayama, Japan) were included in this study. The inclusion criteria for patients and tissue samples are according to a previous report [17]. Briefly, normal kidney and adjacent RCC tissues were collected from patients with 1) no history of neoadjuvant drug therapy or radiotherapy, 2) no history of other tumors, and 3) detailed clinicopathological data. Detailed patient information is listed in Online Resource 1. Genomic DNA and total RNA were extracted from each tissue using TRIzol® reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instruction. Cancer-specific survival (CSS), which is defined as cancer-related death, and overall survival (OS), both of which were calculated from the surgery date to that on which confirmation was obtained, were ascertained from electronic medical records or through a phone call.

Genotyping of *UGT1A6*, *UGT1A9*, and *UGT2B7* variants

Three variants, rs2070959 (541A>G, T181A) of *UGT1A6*, rs2741049 (i399C>T) of *UGT1A9*, and rs7668258 (-161C>T) of *UGT2B7*, were selected as representatives for this study based on the criteria that 1) the reported minor allele frequency is > 0.2 in Japanese or Asian populations, 2) basic functional analysis and clinical studies on the variant have been conducted, and 3) high linkage disequilibrium has been observed with other variants. Genotyping was carried out using the PCR-restriction fragment length polymorphism (RFLP) method with specific primers and restriction enzymes listed in Online Resource 2 with reference to other studies [18, 19],

except for a variant in the *UGT1A9* gene, which was originally designed for this study from NG_002601.2. The typical band patterns for the PCR–RFLP are shown in Online Resource 3. PCR was caried out in a 25 μL reaction mixture containing template genomic DNA isolated from normal kidney tissues, *Ex Taq* HS® (Takara, Shiga, Japan), 1.5 mM $MgSO_4$, 0.2 mM dNTPs, and 0.3 mM of each primer. The PCR conditions were initial denaturation at 94 °C for 3 min, followed by 35 or 37 cycles of denaturation at 94 °C for 20 s, annealing at 58 °C for 15 s, and extension at 72 °C for 10 s.

Microarray dataset analysis

A microarray dataset of GSE40435, including 101 normal kidney and the matched RCC tissues from the Gene Expression Omnibus (GEO) database at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/geo/), were downloaded. The difference in the mRNA expression levels of four nuclear receptors, namely, NR1I2 (pregnane X receptor, PXR), NR1I3 (constitutive androstane receptor, CAR), NR1C1 (peroxisome proliferator-activated receptor alpha, PPARα), and NR2A1 (hepatocyte nuclear factor 4α, HNF4α), between normal kidney and RCC tissues, as well as correlations among the mRNA expressions of UGT1A6, UGT1A9, UGT2B7, and these nuclear receptors, were examined.

Cell culture

The human RCC cell line Caki-1 was cultured in Dulbecco's modified Eagle medium (Merck Japan, Tokyo, Japan) containing 10% fetal bovine serum (Biowest, Bradenton, FL) and 100 U/mL penicillin + 100

131 μg/mL streptomycin (FUJIFILM, Tokyo, Japan) in a humidified 5% CO_2 incubator. The Caki-1 cells were
132 plated at 1.5×10^5 cells/well in a 12-well plate. After 24 h of culturing, test compounds were added, and the
133 cells were cultured at desired periods of time. For RNA sample preparation, TRIzol® reagent was used in a
134 manner similar to that of tissue samples. For protein sample preparation, the cells were collected and suspended
135 in an extraction buffer containing 20 mM tris–HCl (pH 7.4), 150 mM sodium chloride, 10 mM EDTA, 0.5%
136 Triton X-100, and 0.5% sodium cholate. After freezing and thawing twice, the suspended cells were centrifuged
137 at 14,000 rpm twice, and the supernatant, containing the protein samples, was used. Protein concentrations were
138 determined using a BCA Protein Assay Reagent Kit (Pierce, Rockford, IL) according to the manufacturer's
139 protocol.
140
141 **Quantification of mRNA expressions**
142 Real-time reverse transcription PCR was conducted using ReverTra Ace® qPCR RT Master Mix with
143 gDNA Remover (TOYOBO, Osaka, Japan) and THUNDERBIRD® SYBR qPCR Mix (TOYOBO) using specific
144 primers listed in Online Resource 2 with reference to other studies [16, 20]. *GAPDH* mRNA expression was used
145 as an internal standard reference for each mRNA expression.
146
147 **Western immunoblot analysis**
148 Protein samples (10 μg) were separated using a 9.0% (w/v) sodium dodecyl sulfate polyacrylamide
149 gel and transferred onto a polyvinylidene difluoride membrane. The membranes were blocked by incubation

with 3.0% (w/v) skim milk in tris-buffered saline for 1 h at room temperature and incubated with a polyclonal rabbit anti-UGT2B7 antibody (Proteintech, Rosemont, IL; 1:500) in Can Get Signal® Immunoreaction Enhancer Solution (TYOBO) for 12 h at 4 °C. The membrane was then incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG (Cell Signaling Technology, Danvers, MA; 1:5,000) for 1 h at room temperature. For internal standard detection, the membranes were incubated with a polyclonal rabbit anti-β-actin antibody (Proteintech; 1:3,000) for 1 h at room temperature. The protein bands were visualized using a Western Lightning® ECL Pro (PerkinElmer, Waltham, MA).

Statistical analysis

Statistical analyses were performed using Prism 5 (GraphPad, San Diego, CA) and JMP® 15 (SAS Institute Inc., Cary, NC). Paired Student's *t*-test for the comparison of two groups, Pearson's correlation coefficient for the correlation between two variables, and one-way ANOVA followed by Tukey's post hoc test for multiple comparisons were applied. Each genotype was divided into two groups according to the number of samples, as the effects of each genetic variant were not defined. The expression levels of each UGT mRNA were divided into two groups (high or low expression), according to the median mRNA expression value. Associations between clinical characteristics and information on each genotype or mRNA expression were evaluated using the chi-square or Fisher's exact tests. Survival curves were drawn using the Kaplan–Meier method, and differences in survival rates were compared using the log-rank test. Univariate and multivariate Cox analyses were performed to assess significance of prognostic factors. All tests were two-tailed, and $P < 0.05$ indicated statistical significance.

Results

UGT1A6, UGT1A9, and UGT2B7 mRNA expression profiles in normal kidney and RCC tissues

The mRNA expression profiles of UGT1A9, UGT1A6, and UGT2B7 combined with information on their variants (designated as *UGT1A6* 541A>G, *UGT1A9* i399C>T, and *UGT2B7* -161C>T) were examined in normal kidney and RCC tissues. The allele frequencies were 0.20 for *UGT1A6* 541A>G, 0.70 for *UGT1A9* i399C>T, and 0.30 for *UGT2B7* -161C>T and in Hardy–Weinberg equilibrium within the cohort for this study. In normal kidney tissues, the expression levels of the respective UGT mRNA were not affected by the *UGT1A6* 541A>G or *UGT1A9* i399C>T variants (Fig. 1a and 1b). However, UGT2B7 mRNA expression in normal kidney tissues homozygous for *UGT2B7*-161C>T differed considerably from normal kidney tissues homozygous for the wild-type allele (Fig. 1c). The expression levels of the respective UGT mRNA in RCC tissues did not vary with the genotype of the three variants (Fig. 1d–1f).

The mRNA expression levels of *UGT1A9, UGT1A6,* and *UGT2B7* were significantly lower in RCC tissues than in normal kidney tissues (Fig. 1g–1i). *UGT1A9* and *UGT1A6* mRNA expression was downregulated in 72.8% and 60.8% of the RCC tissues, respectively, compared with those in normal kidney tissues (Fig. 1j and 1k). Notably, *UGT2B7* mRNA expression was downregulated in 90.4% of the RCC tissues compared with that in normal kidney tissues (Fig. 1l). Protein expression of UGT2B7, as well as its mRNA expression, in RCC tissues dramatically decreased compared with that in normal kidney tissues (Online Resource 4).

Correlation between UGT1A6, UGT1A9, and UGT2B7 variants, or their mRNA expression, and

clinicopathological parameters in patients with RCC

UGT1A6, UGT1A9, and UGT2B7 variants, and their mRNA expression, were evaluated to assess their correlation with clinicopathological parameters in patients with RCC (Tables 1 and 2). None of the variants correlated with clinicopathological parameters. However, *UGT1A6* mRNA expression was significantly correlated with sex and metastasis in patients with RCC, whereas *UGT1A9* mRNA expression was significantly correlated with age and histological type in patients with RCC. Meanwhile, no clinicopathological parameters were correlated with *UGT2B7* mRNA expression. Additionally in normal kidney tissues, only *UGT2B7* mRNA expression was considerably correlated with patient age (Online Resource 5).

Effect of UGT1A6, UGT1A9, and UGT2B7 variants, and their mRNA expression, on the clinical outcome in patients with RCC

UGT1A6, UGT1A9, and UGT2B7 variants, and their mRNA expression, were evaluated to assess correlations with clinical outcome in patients with RCC. *UGT1A6* 541A>G and *UGT1A9* i399C>T variants were not associated with CSS or OS; however, *UGT2B7*-161C>T was significantly associated with prolonged CSS, but not OS (Fig. 2a–2f). *UGT1A6* and *UGT1A9* mRNA expression was not associated with CSS and OS; nonetheless, patients who had RCC tissues with high *UGT2B7* mRNA expression tended toward significantly prolonged CSS as well as exhibited significantly prolonged OS (Fig. 2g–2l). Meanwhile, no correlation was observed between the mRNA expression of any UGT in normal kidney tissues and patient outcomes (Online Resource 6).

The prognostic significance of UGT1A6, UGT1A9, and UGT2B7 variants, and their corresponding

mRNA expression, in patients with RCC are presented in Table 3. Using univariate Cox analysis, *UGT2B7* -161C>T variant was identified as a significant prognostic risk factor for CSS, whereas the *UGT2B7* mRNA expression level was identified as a significant prognostic risk factor in OS. Based on the results of univariate analysis and number of events in the present study, three variables for CSS (TNM stage, presence of *UGT2B7*-161C>T variant, and *UGT2B7* mRNA expression level), and two variables for OS (TNM stage and *UGT2B7* mRNA expression level) were further investigated using multivariate Cox analysis. The results showed that the presence of *UGT2B7*-161C>T variant and *UGT2B7* mRNA expression level in patients with RCC represented significant independent prognostic factors for CSS and CSS/OS, respectively.

Correlations among UGT1A6, UGT1A9, UGT2B7, and nuclear receptor expression in RCC

Correlations among *UGT1A6, UGT1A9,* and *UGT2B7* mRNA expression in normal kidney and RCC tissues were examined in the study cohort (Fig. 3a). In normal kidney tissues, *UGT1A9* mRNA expression significantly correlated with those of *UGT1A6* and *UGT2B7* mRNA. In RCC tissues, the mRNA expression of all UGTs significantly correlated with each other.

The transcriptional activity of UGT is reportedly regulated by several nuclear receptors, including PXR, CAR, PPARα, and HNF4α [21-23]. To assess the possible mechanism underlying the downregulation of *UGT1A6, UGT1A9,* and *UGT2B7* mRNA expression in RCC tissues, the mRNA expression of these four nuclear receptors was examined in RCC tissues using a dataset from the GEO database. The mRNA expression levels of three receptors, namely, *NR1I3, NR1C1,* and *NR2A1,* in RCC tissues were significantly lower than those in normal

kidney tissues (Fig. 3b). Moreover, the mRNA expression levels of *UGT1A6*, *UGT1A9*, and *UGT2B7* significantly correlated with those of *NR1C1* and *NR2A1* (Fig. 3c).

To assess whether the correlation between UGT2B7, whose mRNA expression was identified as a significant prognostic factor for RCC in this study, and HNF4α or PPARα in the GEO database is reflected *in vitro*, a cell-based assay was performed using agonists for these two nuclear receptors. Clofibrate is a traditionally well-known strong PPARα agonist. Although HNF4α is an orphan nuclear receptor in that a corresponding ligand has not yet been definitively identified, alverine is reported to possess HNF4α agonistic properties [24]. Thus, changes in UGT2B7 expression were determined after the addition of these two drugs as agonists for PPARα or HNF4α. Caki-1 cell line was selected because the cells are expected to maintain the gene expression of UGT2B7, PPARα, and HNF4α from gene expression data in the CCLE database (The Cancer Cell Line Encyclopedia, https://sites.broadinstitute.org/ccle/) and was confirmed to demonstrate these mRNA expressions using RT–PCR (data not shown). *UGT2B7* mRNA expression, as well as its corresponding protein expression, remained unchanged after alverine addition; however, it was significantly increased by the presence of clofibrate at a high concentration (100 μM) (Fig. 3d and 3e).

Discussion

Despite the liver generally being the primary focus in UGT research (being its primary expressor), in this study, we provide new detailed insights on the clinical relevance of *UGT* variants and their mRNA expression profiles in the kidneys of patients with RCC. In the present study, we focused on three *UGT* variants, *UGT1A6*, *UGT1A9*, and *UGT2B7*. The UGT mRNA and protein subtypes expressed in the normal kidney and RCC tissues are similar [16]. Although mRNA of other UGTs, such as *UGT1A1*, *UGT1A7*, and *UGT2B11*, are detectable in normal kidney and RCC tissues, their expression levels are far lower than those of *UGT1A6*, *UGT1A9*, and *UGT2B7* [14, 16]. Therefore, the significant UGT subtypes in the kidneys are *UGT1A6*, *UGT1A9*, and *UGT2B7*, while the other UGTs probably do not have a significant impact on renal cell abundance and function. Moreover, a significant correlation has been described between the expression of these three UGT mRNAs and their protein expressions, as well as between their protein abundance and metabolic activities for typical substrates in RCC tissues [16]. These reports indicate that the mRNA expression levels of *UGT1A6*, *UGT1A9*, and *UGT2B7* strongly reflect both their protein abundance and metabolic capacities in RCC tissues.

The allele frequencies of the three variants, *UGT1A6* 541A>G, *UGT1A9* i399C>T, and *UGT2B7* -161C>T, in our cohort were in concordance with previous reports at 0.22, 0.64, and 0.27, respectively [25-27]. Several linkage disequilibria were observed, and several haplotypes in the *UGT* genes have been suggested. In addition, all three variants examined here have high linkage disequilibrium with several variants not only in the coding region but also in the promoter and intron regions; these disequilibria can further provide insights into other *UGT* genes [25-30]. For instance, we found that the *UGT2B7*-161C>T and *UGT1A6* 541A>G variants

exhibited high linkage disequilibrium with the *UGT2B7* 802C>T (rs7439366, H268Y) and *UGT1A9* i399C>T variants, respectively (data not shown). Hence, we sought to evaluate mRNA expression, including the variants observed in the coding region, that do not independently appear to affect their mRNA expression levels. Our data suggest that two variants, *UGT1A6 541A*>G and *UGT1A9* i399C<T, do not affect expression levels of the respective UGT mRNA in normal kidney or RCC tissues. Nonetheless, the mRNA expression of UGT2B7 was altered by the *UGT2B7* -161C>T variant in only normal kidney tissues. The discrepancy between normal kidney and RCC tissues may be partially explained by the downregulation of *UGT2B7* mRNA expression in RCC tissues compared with that in normal kidney tissues. Nevertheless, the three variants examined in the present study do not impact their mRNA expression levels in RCC tissues.

The mRNA expression levels of all three renal UGTs, *UGT1A6*, *UGT1A9*, and *UGT2B7*, were downregulated in RCC tissues. In particular, a marked decrease was observed in *UGT2B7* mRNA expression in >90% of RCC tissues. The significant correlation among the mRNA expression levels of *UGT1A6, UGT1A9*, and *UGT2B7* indicate that their expression may be regulated by similar mechanisms in RCC tissues. The four nuclear receptors examined in the present study, PXR, CAR, PPARα, and HNF4α (all of which are expressed in the kidney) [31-34], have been suggested to regulate the transcriptional activity of renal UGT. Although the mRNA expression levels of three of these four receptors, as well as UGTs, in RCC tissues were decreased compared with those in normal kidney tissues, no correlation was detected between the mRNA expression levels of CAR and any UGT. Moreover, our cell-based assay elucidated that the PPARα agonist, clofibrate, induced UGT2B7 expression in an RCC-derived cell line. Although it remains unknown whether HNF4α stimulation could induce UGT2B7

expression and more detailed assessments, such as reporter gene assay, are essential to ascertain this finding, PPARα, at least, is thought to be involved in the downregulation of UGT2B7 in RCC.

The mRNA expression levels of *UGT1A6* and *UGT1A9* are modulated by age and sex [35-37], and they correlate with several clinicopathological parameters of patients with RCC in the present study. However, lack of correlation of *UGT1A6* 541A>G and *UGT1A9* i399C>T variants, or their mRNA expression, with survival of patients with RCC indicates that most likely they do not impact patient outcomes. Meanwhile, *UGT2B7*-161C>T and its mRNA expression level correlate with outcomes of patients with RCC. Nonetheless, it remains unclear why a correlation was not observed between *UGT2B7*-161C>T and OS. Nevertheless, these findings indicate that UGT2B7 impacts the outcomes for patients with RCC. Low mRNA expression of *UGT2B7*, caused by the accompanying downregulation of nuclear receptors, may worsen the outcome for patients with RCC. Although the presence of homozygous *UGT2B7*-161C>T variant did not alter *UGT2B7* mRNA expression levels in RCC tissues, its high linkage disequilibrium in the coding region, with other variants such as 802C>T, causes a nonsynonymous change (H268Y). Hence, the expression level of *UGT2B7* and its enzymatic activity may be involved in the outcome of patients with RCC. The fact that these two variables were identified as independent prognostic factors in the present study may support this explanation.

As this study focuses on clinical investigations using biopsied kidney tissues, detailed basic analyses of the intricate role of UGT2B7 in RCC cells were not carried out, which may be considered a limitation of the present study. There are two potential roles of UGT2B7 in RCC progression: 1) drug resistance via metabolism in anticancer agents and 2) progressive regulation via changing levels of endogenous substrates [3]. However, the

299 role of UGT2B7 in RCC may be independent of drug resistance as the outcome in patients with RCC with high
300 *UGT2B7* mRNA expression was superior to that for patients with low expression. Moreover, no correlation was
301 observed between the expression level of *UGT2B7* mRNA and response rate of anticancer agents in the small
302 number of sample tissues (n = 15, data not shown). Meanwhile, a recent study showed that *UGT2B7* expression is
303 downregulated and the endogenous carcinogenic catechol substrates of UGT2B7 accumulate in endometrial
304 cancer [9]. Importantly, the *UGT2B7* 802C>T variant that shows high linkage disequilibrium with
305 *UGT2B7*-161C>T decreased the risk of endometrial cancer, suggesting that this variant might increase enzymatic
306 activity. Regarding specific endogenous substrates to RCC, 20-hydroxyeicosatetraenoic acid, a substrate of
307 UGT2B7, stimulates the proliferation of RCC *in vitro* and *in vivo* [38, 39]. Taken together, low expression and
308 enzymatic activity of UGT2B7 may contribute to RCC progression via accumulation of endogenous substrates
309 that can stimulate cancer cells.
310

Conclusion

This study determined the expression profiles and clinical relevance of *UGT1A6*, *UGT1A9*, and *UGT2B7* variants in RCC. The variant *UGT2B7* and its mRNA expression level correlated with the outcomes of patients with RCC, suggesting that UGT2B7 may have an important role in RCC. UGT2B7, therefore, has implications as a potential promising marker for prognostication in patients with RCC. The findings of the present study provide basic information on UGT expression in the kidney and may facilitate an improved understanding on the importance of UGTs in RCC.

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Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences of Okayama University Hospital, Japan (February 14, 2018/No. 1802–033).

Consent to Participate: Patients provided informed consent for participating in the study and were given the opportunity to opt out of this study at their will.

Consent to Publish: Not applicable.

Figure Legends

Fig. 1 Expression of *UGT1A6, UGT1A9*, and *UGT2B7* mRNAs in normal kidney and RCC tissues. Differences in the mRNA expression of *UGT1A6* (**a**), *UGT1A9* (**b**), and *UGT2B7* (**c**) in normal kidney tissues among their genetic variants (541A>G for *UGT1A6*, i399C>T for *UGT1A9*, and -161C>T for *UGT2B7*). Differences in the mRNA expression of *UGT1A6* (**d**), *UGT1A9* (**e**), and *UGT2B7* (**f**) in RCC tissues among their genetic variants. Differences in the mRNA expression of *UGT1A6* (**g**), *UGT1A9* (**h**), and *UGT2B7* (**i**) between normal kidney (N) and RCC tissues (T). Wd, homozygous of wild-type; het, heterogenous of variant-type; mut, homozygous of variant-type. Expression levels are presented as the ratio of the average value of each mRNA expression with respect to that in normal tissues [mean ratio (line below x-axis labels) ± SE (value in parentheses)]. Detailed mRNA expression changes of *UGT1A6* (**j**), *UGT1A9* (**k**), and *UGT2B7* (**l**) from normal kidney to RCC tissues. Expression change is presented as the ratio of the mRNA expression level in normal tissues with that in RCC tissues (logarithmic scale). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001

Fig. 2 Impact of *UGT1A6, UGT1A9,* and *UGT2B7* mRNA expression and their variants on the survival of patients with RCC. Kaplan–Meier curves of CSS in 541A>G for *UGT1A6* (**a**), i399C>T for *UGT1A9* (**b**), and -161C>T for *UGT2B7* (**c**) and of overall survival (OS) in 541A>G (**d**), i399C>T (**e**), and -161C>T (**f**). Kaplan–Meier curves of cancer-specific survival (CSS) in *UGT1A6* (**g**), *UGT1A9* (**h**), and *UGT2B7* (**i**) mRNA expression and of OS in *UGT1A6* (**j**), *UGT1A9* (**k**), and *UGT2B7* (**l**) mRNA expression

Fig. 3 Correlations among *UGT1A6, UGT1A9, UGT2B7,* and nuclear receptor mRNA expression in RCC tissues. (**a**) Correlation in terms of the Pearson's correlation coefficient for *UGT1A6, UGT1A9*, and *UGT2B7* mRNA expression in the study cohort. (**b**) Differences in the mRNA expression of *NR1I2, NR1I3, NR1C1*, and *NR2A1* between normal kidney (N) and RCC tissues (T) in a dataset obtained from the GEO database. The expression levels are presented as the ratio of the average value of each mRNA expression with respect to normal tissues and are presented as mean ± SE. (**c**) Correlations in terms of Pearson's correlation coefficients of *UGT1A6, UGT1A9, UGT2B7,* and nuclear receptor mRNA expression in RCC tissues in a dataset obtained from the GEO database. (**d**) Changes in *UGT2B7* mRNA expression upon the addition of the HNF4α and PPARα agonists, alverine and clofibrate, respectively. Total RNA was extracted from cells 12 h after addition of these compounds. (**e**) Changes in UGT2B7 protein expression upon addition of the HNF4α and PPARα agonists, alverine and clofibrate, respectively. Proteins were extracted from cells 24 h after addition of these compounds. The desired periods of time for culturing were determined based on cell growth and toxicity of test compounds. C, control (0.5% dimethyl sulfoxide); PC, positive control. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$