ANTICANCER RESEARCH *41*: 2511-2521 (2021)
doi:10.21873/anticanres.15029

Relevance of CYP3A5 Expression on the Clinical Outcome of Patients With Renal Cell Carcinoma

JUN MATSUMOTO[1], YUMI KOTERA[1], SHOGO WATARI[2], KOICHI TAKEUCHI[1], HIDEO UEKI[2], TOSHIHIRO KOYAMA[3], KOICHIRO WADA[2], MASACHIKA FUJIYOSHI[1], YASUTOMO NASU[2] and NORITAKA ARIYOSHI[1]

[1]*Department of Personalized Medicine and Preventive Healthcare Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan;*
[2]*Department of Urology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan;*
[3]*Department of Pharmaceuticals Biomedicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan*

Abstract. *Background/Aim: This study aimed to elucidate the detailed characteristics of CYP3A5 expression and the association between CYP3A5 expression and clinical outcomes in patients with renal cell carcinoma (RCC). Patients and Methods: This study retrospectively enrolled 124 Japanese patients with RCC treated at the Okayama University Hospital. The commonest CYP3A5 gene polymorphism, CYP3A5*3, and expression levels of CYP3A5 mRNA and protein in each tissue were examined. Results: Expression of CYP3A5 mRNA and protein in RCC tissues was significantly down-regulated compared to that in adjacent normal tissues. High level of CYP3A5 mRNA expression significantly extended cancer-specific survival (p=0.004) and overall survival (p=0.002). The CYP3A5 mRNA expression level was identified as a significant independent prognostic factor for both cancer-specific survival and overall survival. Conclusion: CYP3A5 could serve as a potential marker for prognostication and treatment planning for patients with RCC.*

Renal cell carcinoma (RCC) is the commonest cancer of the kidney and comprises approximately 90% of all cases of kidney cancer (1). RCC is estimated to account for 2% of new cancer cases or cancer deaths worldwide (2, 3). Advances in cancer therapy have improved the 5-year survival rates for RCC, although the overall prognosis for RCC remains unsatisfactory, at approximately 65% of the current 5-year survival rates for RCC (4). Moreover, the global incidence of RCC has been gradually increasing (5).

Cytochrome *P450* (CYP) 3A is the most abundant CYP subfamily that is responsible for the metabolism of a large number of substrates in humans. The major CYP3A isoform is CYP3A4; however, three minor isoforms, CYP3A5, CYP3A7, and CYP3A43, have been reported (6). Of these, CYP3A5 is the most abundantly expressed enzyme of the minor CYP3A isoforms in adults (6). The *CYP3A5* gene is highly polymorphic, which causes individual variations in the expression of CYP3A5. The commonest allele responsible for the variable protein expression of CYP3A5 is *CYP3A5*3* (rs776746, 6986 A>G) (7). Individuals who are homozygous for the *CYP3A5*3* allele either express very low levels of CYP3A5 protein or lack CYP3A5 protein expression (7, 8). Interestingly, evidence from small cohort studies indicate that CYP3A5, but not CYP3A4, is markedly expressed in the normal kidney with a *CYP3A5*1* allele (9, 10). Thus, CYP3A5 is considered to play a role in substrate metabolism in the kidney.

Most studies on the role of CYP3A5 in cancer have focused on the metabolism of exogenous agents, such as anticancer drugs, in the liver and small intestine. Many anticancer drugs are reported to be candidate substrates of CYP3A5 (11, 12). In addition, evidence has shown that CYP3A5 has several roles in cancer progression, via the metabolism of endogenous or carcinogenic substrates, that are independent of the metabolism of anticancer drugs (13).

Correspondence to: Jun Matsumoto (ORCID iD: 0000-0001-7224-108X), PhD, Department of Personalized Medicine and Preventive Healthcare Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan. Tel and Fax: +81 862357873, e-mail: matsumotoj@okayama-u.ac.jp

Key Words: CYP3A5 protein, human, cytochrome P450 CYP3A, renal cell carcinoma treatment outcome.

Moreover, recent studies have suggested that CYP3A5 itself acts as a tumor suppressor by regulating cell signaling (14, 15). Thus, CYP3A5 expression has been proposed as a potential marker for cancer prevention and treatment. However, the potential role of CYP3A5 in promoting or inhibiting cancer progression may differ depending on the type of cancer. Thus far, the detailed characteristics of CYP3A5 expression in RCC and its clinical relevance in RCC patients remain unknown despite the fact that renal cells express CYP3A5. More information on CYP3A5 expression profiles in RCC may contribute to a better understanding of the underlying malignant behavior and metabolic capacity of RCC.

The aim of this study was to elucidate the detailed expression profiles of CYP3A5 and the association between CYP3A5 expression and clinical outcomes in RCC patients. Unlike most previously reported studies that focused on either the genotype or phenotype of CYP3A5 in cancer patients, this study has clarified both genotypic and phenotypic characteristics in RCC tissues. Moreover, to the best of our knowledge, this study includes the largest number of samples to directly elucidate the details of CYP3A5 expression profiles and its clinical relevance in RCC patients.

Patients and Methods

Patients. This study retrospectively enrolled 124 Japanese RCC patients who underwent surgery between March 2003 and December 2015 at the Okayama University Hospital and fulfilled the following criteria: 1) no neoadjuvant drug therapy or radiotherapy, 2) no history of other tumors, and 3) availability of detailed clinicopathological data. The collected RCC tissues and the corresponding adjacent normal kidney tissue specimens were stored at −80˚C until analysis. Detailed information on patient characteristics is listed in the Table I. The nuclear grade values of participants were determined according to the General Rule for Clinical and Pathological Studies on Renal Carcinoma (16). In the samples with Fuhrman's grading score, grades 1 to 3 corresponded to Fuhrman's grading, and Fuhrman's grade 4 corresponded to grade 3 disease scoring by the general rule. Cancer-specific survival (CSS), which is defined as death due to cancer-related problems, and the overall survival (OS) was ascertained from the records at Okayama University Hospital or the Okayama prefectural office or through a phone call to the patient, other hospitals, or the relevant general practitioner. This study was approved by the ethics committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences of Okayama University Hospital (approval number: 1802–033) and was conducted in accordance with the ethical principles stated in the Declaration of Helsinki. Patients provided informed consent for study participation and were given the opportunity to freely opt out of this study at any time.

Preparation of DNA, RNA, and protein from each tissue specimen. RCC tissues or the adjacent normal tissues were sliced in half, and each part was used separately for genomic DNA/total RNA isolation and protein extraction, respectively. When the weight of tissue specimens was less than 30 mg, the tissues were not divided and only genomic DNA/total RNA was isolated without protein extraction. Genomic DNA and total RNA isolation were carried out using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. Total proteins from each tissue were extracted by homogenizing with an extraction buffer comprising 20 mM Tris-HCl (pH 7.4), 150 mM sodium chloride, 10 mM EDTA, 0.5% Triton X-100, and 0.5% sodium cholate, followed by two cycles of freezing and thawing. After centrifugation for 15 min at 14,000 rpm at 4˚C, the supernatant was used as the protein fraction.

Table I. *Demographics and clinical characteristics of the study participants*.

Characteristics	Patient number (n=124)	(Range or %)
Age, years (mean±SD)	64.19±11.55	(27 to 87)
Gender (male/female)	77/47	(62.1/37.9)
Tumor stage (T1/T2/T3)	77/12/35	(62.1/9.7/28.2)
Lymph node status (N0/N1/N2)	115/3/6	(92.7/2.5/4.8)
Metastasis (M0/M1)	104/20	(83.9/16.1)
TNM stage (I/II/III/IV)	74/11/19/20	(59.7/8.9/15.3/16.1)
Nuclear grade (1/2/3)	22/71/31	(17.7/57.3/25.0)
Histological type (clear cell/papillary/ chromophobe/mix or others)	98/6/10/10	(79.0/4.8/8.1/8.1)

*CYP3A5*3 genotyping*. *CYP3A5*3* genotyping for each patient was carried out by the PCR-restriction fragment length polymorphism method with DraI (Takara, Shiga, Japan) and using specific primers (forward, 5'-CTAACCATAATCTCTTTTAAGAGCTCTTTTGTC TTTAA-3'; reverse, 5'-ACTTTGATCATTATGTTATGTAATCCA TAC-3') as described previously (17). Genomic DNA isolated from adjacent normal kidney tissues was used as the template for *CYP3A5*3* genotyping.

Real-time PCR. Real-time reverse transcription PCR was carried out using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka, Japan) and THUNDERBIRD® SYBR qPCR Mix (TOYOBO) with specific primers (forward, 5'-AAGTATGGAAAAAT GTGGGGAAC-3'; reverse, 5'-CTGTAGGCCCCAAAGATGTC-3') according to the manufacturer's instructions as described previously (17). GAPDH mRNA expression was used as an internal standard reference for CYP3A5 mRNA expression.

Western immunoblot analysis. Western immunoblot analysis was carried out as described previously (17). Polyclonal rabbit antibodies against CYP3A5 (Abcam, Cambridge, England) and GAPDH (Proteintech, Rosemont, IL, USA) were used as the primary antibodies. Recombinant CYP3A5 (Corning Gentest, Woburn, MA, USA) was used as a positive control for the specific detection of CYP3A5. GAPDH protein expression was used as an internal standard reference for CYP3A5 protein expression.

Microarray dataset analysis. Microarray datasets from the Gene Expression Omnibus (GEO) database at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/geo/)

were used to determine the difference in the expression levels of CYP3A5 mRNA between normal kidney tissues and RCC tissues. The database included only two datasets from very small cohorts (GSE781 and GSE6344) that contained paired data for RCC tissues and adjacent normal tissues.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 5 (GraphPad, San Diego, CA, USA) and JMP® 15 (SAS Institute Inc., Cary, NC, USA). Paired Student's *t*-test or Mann–Whitney *U*-test test were used for the comparison of the means of the two groups. One-way ANOVA followed by Tukey's post hoc test was used for multiple comparisons. The cutoff value for CYP3A5 mRNA expression was selected by using receiver operating characteristic curve analysis, and the highest value with both maximum sensitivity and specificity on the curve for survival of patients was applied. Associations between clinical characteristics and information on CYP3A5 expression were evaluated using the chi-square or Fisher's exact test. Survival curves were produced 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 identify independent prognostic factors. All tests were two-tailed, and $p<0.05$ indicated statistical significance.

Results

*CYP3A5 expression profiles in RCC tissues and adjacent normal tissue specimens based on CYP3A5*3 genotype*. The expression of CYP3A5 protein was detected in RCC tissues and adjacent normal tissues (Figure 1A). To assess the details of CYP3A5 expression profiles, CYP3A5 mRNA and protein expression levels were analyzed based on the *CYP3A5*3* genotypes (Figure 1B to E). In the adjacent normal tissues, CYP3A5 mRNA expression with the genotypes of **1/*1* and **1/*3* was significantly higher than that of the **3/*3* genotype. In RCC tissues, CYP3A5 mRNA expression with the genotypes **1/*1* and **1/*3* was significantly higher than that with the **3/*3* genotyped; moreover, a significant difference in CYP3A5 mRNA expression between **1/*1* and **1/*3* genotypes was observed. The results for CYP3A5 protein expression profiles were almost the same as those for CYP3A5 mRNA. In the adjacent normal tissues, the protein expression of CYP3A5 with genotypes **1/*1* and **1/*3* was significantly higher than that of the **3/*3* genotype. In RCC tissues, the protein expression of CYP3A5 was significantly higher in **1/*1* than in **1/*3* and **3/*3*; however, there was no significant difference in CYP3A5 protein expression between the **1/*3* and **3/*3* genotypes. Significant correlations were observed among CYP3A5 mRNA expression level and its protein expression level in both adjacent normal tissues and RCC tissues (Table II). The allele frequency of *CYP3A5*3* was 0.79, which was in the Hardy–Weinberg equilibrium. The concordance rates of the *CYP3A5*1* genotype with the CYP3A5 protein expression in the adjacent normal tissues and RCC tissues were 86.4% (n=88) and 70.2% (n=104), respectively.

Difference in the level of CYP3A5 expression between RCC tissues and the adjacent normal tissues. In two datasets (GSE781, n=7; CSE6344, n=10) in the GEO database, the expression level of CYP3A5 mRNA in RCC tissues tended to be or was significantly lower than that in the adjacent normal tissues (Figure 2A and B). These results from the two datasets were in accordance with those from the cohort of this study. The expression level of CYP3A5 mRNA in RCC tissues was significantly lower than that in the adjacent normal tissues (Figure 2C). With regard to the level of CYP3A5 protein expression, there was a significant difference in the level of CYP3A5 protein expression between RCC tissues and adjacent normal tissues (Figure 2D). Frequent down-regulation of CYP3A5 protein expression was observed in RCC tissues compared with that in the adjacent normal tissues (Figure 2E).

Correlation between CYP3A5 expression and clinicopathological parameters in RCC patients. From results of the significant correlation between CYP3A5 mRNA and its protein expressions and low number of patients who expressed the CYP3A5 protein due to down-regulation of CYP3A5 protein expression, several clinicopathological parameters of RCC patients were assessed for correlation with the CYP3A5 mRNA levels (Table II). In the adjacent normal tissues, CYP3A5 mRNA was not correlated with any clinicopathological parameters. In RCC tissues, high levels of CYP3A5 mRNA expression were significantly correlated with lymph node status and histological types. With regard to the down-regulation of CYP3A5 mRNA expression in RCC tissues, decreased CYP3A5 mRNA expression in RCC tissues was significantly correlated with a higher TNM stage and histological types.

Impact of CYP3A5 expression on clinical outcome in RCC patients. The CYP3A5 mRNA level was evaluated to determine whether they correlated with clinical outcomes in RCC patients (Figure 3). In the adjacent normal tissues, there were no differences in CSS and OS with regard to the levels of CYP3A5 mRNA expression. On the other hand, patients with high CYP3A5 mRNA expression levels in RCC tissues exhibited significantly longer CSS and OS, indicating that the level of CYP3A5 expression possibly correlated with clinical outcome in RCC patients. With regard to changes in the levels of CYP3A5 mRNA expression, decreased CYP3A5 mRNA expression also significantly shortened CSS and OS.

Next, we investigated the prognostic value of the level of CYP3A5 mRNA expression in RCC tissues (Table III). Univariate Cox analysis identified TNM stage as a prognostic risk factor for CSS, whereas level of CYP3A5 mRNA expression tended to be a significant risk factor. On the other hand, TNM stage and the level of CYP3A5 mRNA expression were identified as significant prognostic risk

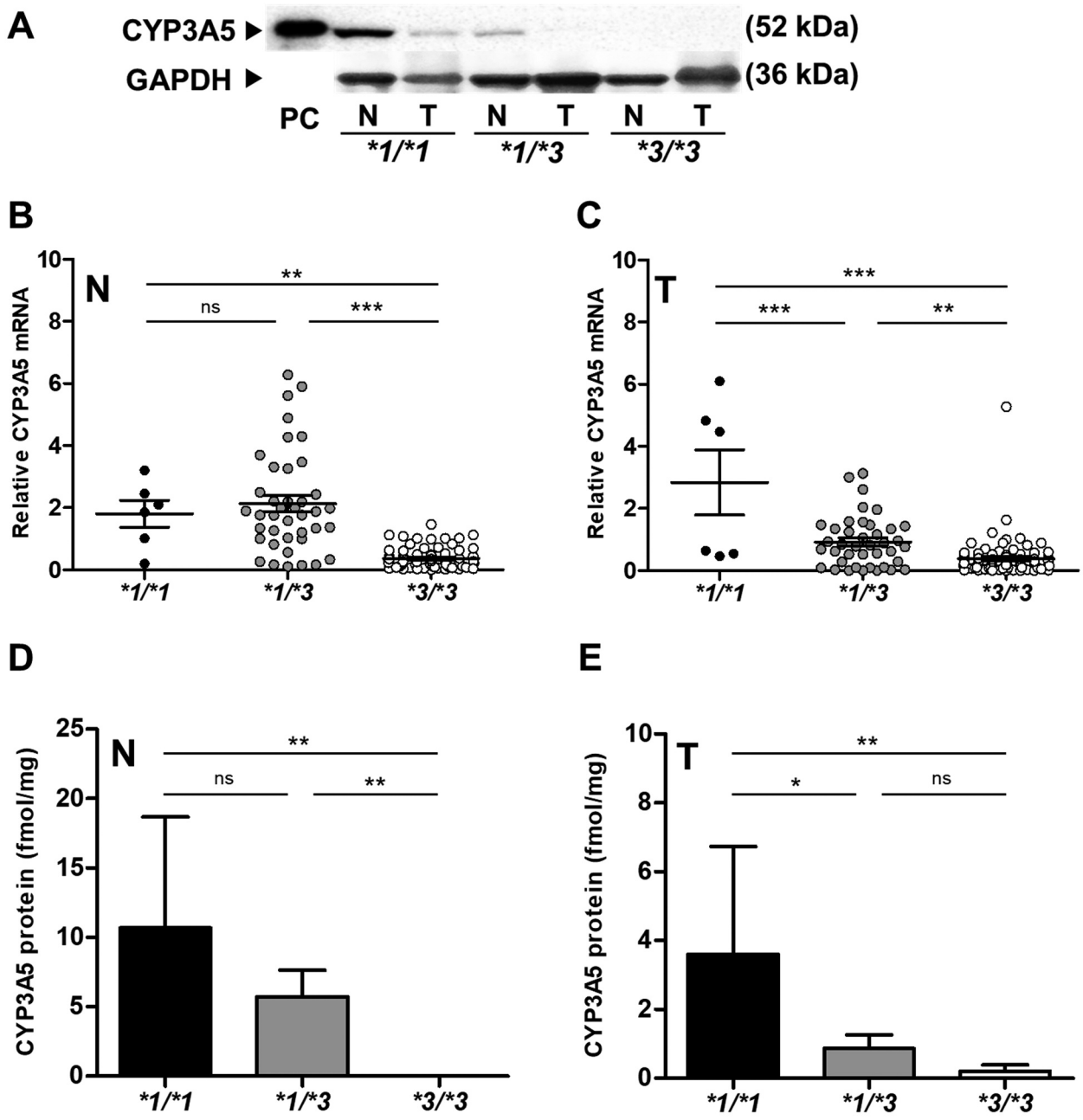

Figure 1. *CYP3A5 mRNA and protein expression based on CYP3A5*3 genotype in RCC tissues and the adjacent normal tissues. (A) Representative pattern of CYP3A5 protein expression in adjacent normal tissues (N) and RCC tissues (T). Proteins (20 μg) were analyzed by Western immunoblot analysis. PC, positive control (0.5 fmol). (B) and (C), the level of CYP3A5 mRNA expression in adjacent normal tissues (N; B) and RCC tissues (T; C). The expression level is presented as the ratio of the average value of CYP3A5 mRNA expression in the adjacent tissues. (D) and (E), the level of CYP3A5 protein expression in adjacent normal tissues (N; D) and RCC tissues (T; E). The expression levels are presented as mean±SD. ***, **, *, and "ns" indicate p<0.001, p<0.01, p<0.05, and not significant, respectively.*

factors for OS in univariate Cox analysis. Based on the results for univariate Cox analysis, the number of events in the present study, and the results from a previous paper from similar cohorts (18), we further analyzed two variables, TNM stage and level of CYP3A5 mRNA expression, in multivariate Cox analysis. The results showed that the level of CYP3A5 mRNA expression was an independent prognostic factor for CSS and OS in RCC patients.

Table II. *The association between CYP3A5 and clinicopathological characteristics.*

Characteristics	*CYP3A5* mRNA in N, n (%)				*p*-Value	CYP3A5 mRNA in T, n (%)				*p*-Value	CYP3A5 mRNA, n (%)				*p*-Value
	High		Low			High		Low			Increasing		Decreasing		
Total number	88					104					124				
CYP3A5 genotype															
*1/*1 and *1/*3	30	(88.2)	4	(7.4)	**<0.001**	33	(41.6)	5	(22.2)	0.060	-	-	-	-	-
*3/*3	4	(11.8)	50	(92.6)		47	(58.4)	19	(77.8)		-	-	-	-	
CYP3A5 protein expression															
Yes	20	(58.8)	2	(3.7)	**<0.001**	11	(13.8)	0	(0.0)	**0.001**	-	-	-	-	-
No	14	(41.2)	52	(96.3)		69	(86.2)	24	(100.0)		-	-	-	-	
Age, years															
<65	18	(52.9)	23	(42.6)	0.343	36	(45.0)	12	(50.0)	0.667	22	(45.8)	35	(46.1)	0.981
≥65	16	(47.1)	31	(57.4)		44	(55.0)	12	(50.0)		26	(54.2)	41	(53.9)	
Gender															
Male	20	(58.8)	33	(61.1)	0.831	51	(63.8)	16	(66.7)	0.793	17	(35.4)	28	(36.8)	0.872
Female	14	(41.2)	21	(38.9)		29	(36.2)	8	(33.3)		31	(64.6)	48	(63.2)	
Tumor stage															
T1	21	(61.8)	34	(63.0)	0.910	50	(62.5)	13	(54.2)	0.148	34	(70.8)	43	(56.6)	0.111
T2+T3	13	(38.2)	20	(37.0)		30	(37.5)	11	(45.8)		14	(29.2)	33	(43.3)	
Lymph node status															
N0	32	(94.2)	51	(94.4)	1.000	76	(95.0)	19	(79.2)	**0.029**	47	(97.9)	68	(89.5)	0.152
N1+N2	2	(5.8)	3	(5.6)		4	(5.0)	5	(20.8)		1	(2.1)	8	(10.5)	
Metastasis															
M0	29	(85.3)	46	(85.2)	1.000	69	(86.3)	17	(70.8)	0.095	43	(89.6)	61	(80.3)	0.169
M1	5	(14.7)	8	(14.8)		11	(13.7)	7	(29.2)		5	(10.4)	15	(19.7)	
TNM stage															
I+II	22	(64.7)	39	(72.2)	0.459	57	(71.3)	13	(54.2)	0.124	38	(79.2)	47	(61.8)	**0.043**
III+IV	12	(35.3)	15	(27.8)		23	(28.7)	11	(45.8)		10	(20.8)	29	(38.2)	
Nuclear grade															
1+2	25	(73.5)	46	(85.2)	0.182	62	(77.5)	15	(62.5)	0.152	9	(18.4)	22	(28.9)	0.202
3	9	(26.5)	8	(14.8)		18	(22.5)	9	(37.5)		39	(81.6)	54	(71.1)	
Histological type															
Clear cell	27	(79.4)	39	(72.2)	0.444	67	(83.8)	12	(50.0)	**0.001**	43	(89.6)	55	(72.4)	**0.022**
Other	7	(20.6)	15	(27.8)		13	(16.2)	12	(50.0)		5	(10.4)	21	(27.6)	

The number of samples was 88 and 104, which corresponded to samples of *CYP3A5*3* genotype, CYP3A5 mRNA, and CYP3A5 protein obtained from adjacent normal tissues and RCC tissues, respectively. CYP, Cytochrome P450; N, adjacent normal tissues; T, RCC tissues. Bold values indicate statistical significance.

Discussion

Currently, CYP3A5 expression in cancer is in the spotlight (14, 15, 19-21). Herein, we first elucidated the detailed expression of CYP3A5 in combination with the *CYP3A5*3* genotypes and CYP3A5 mRNA expression in RCC tissues using the largest number of samples as compared with other papers. In many previous reports, there is no clear evidence of renal CYP3A4 expression (9, 22, 23). In concordance with those results, we did not find a detectable CYP3A4 protein expression in both RCC tissues and the adjacent normal tissues (n=20, data not shown). Thus, the main CYP3A isoform expressed in the kidney is CYP3A5, and not CYP3A4. Therefore, clarifying the detailed expression of CYP3A5 in RCC tissues was valuable in this study.

The allele frequency of *CYP3A5*3* in this study was similar to that in previous reports from the Japanese population (24, 25). Nonetheless, CYP3A5 protein expression did not completely correspond to the *CYP3A5*1* genotype in both the adjacent normal tissues and RCC tissues. The concordance rates of CYP3A5 protein expression with the *CYP3A5*1* genotype in the kidney were lower than those in the liver and small intestine, where CYP3A5 protein expression was almost completely correlated with the *CYP3A5*1* genotype (8). Several polymorphisms, such as *CYP3A5*6* and **7*, in the *CYP3A5* gene that cause variations in the level of CYP3A5 protein expression have been reported (6). However, most of these polymorphisms were not observed or were rarely detected in the Japanese population (25, 26). CYP3A5 is expressed to a

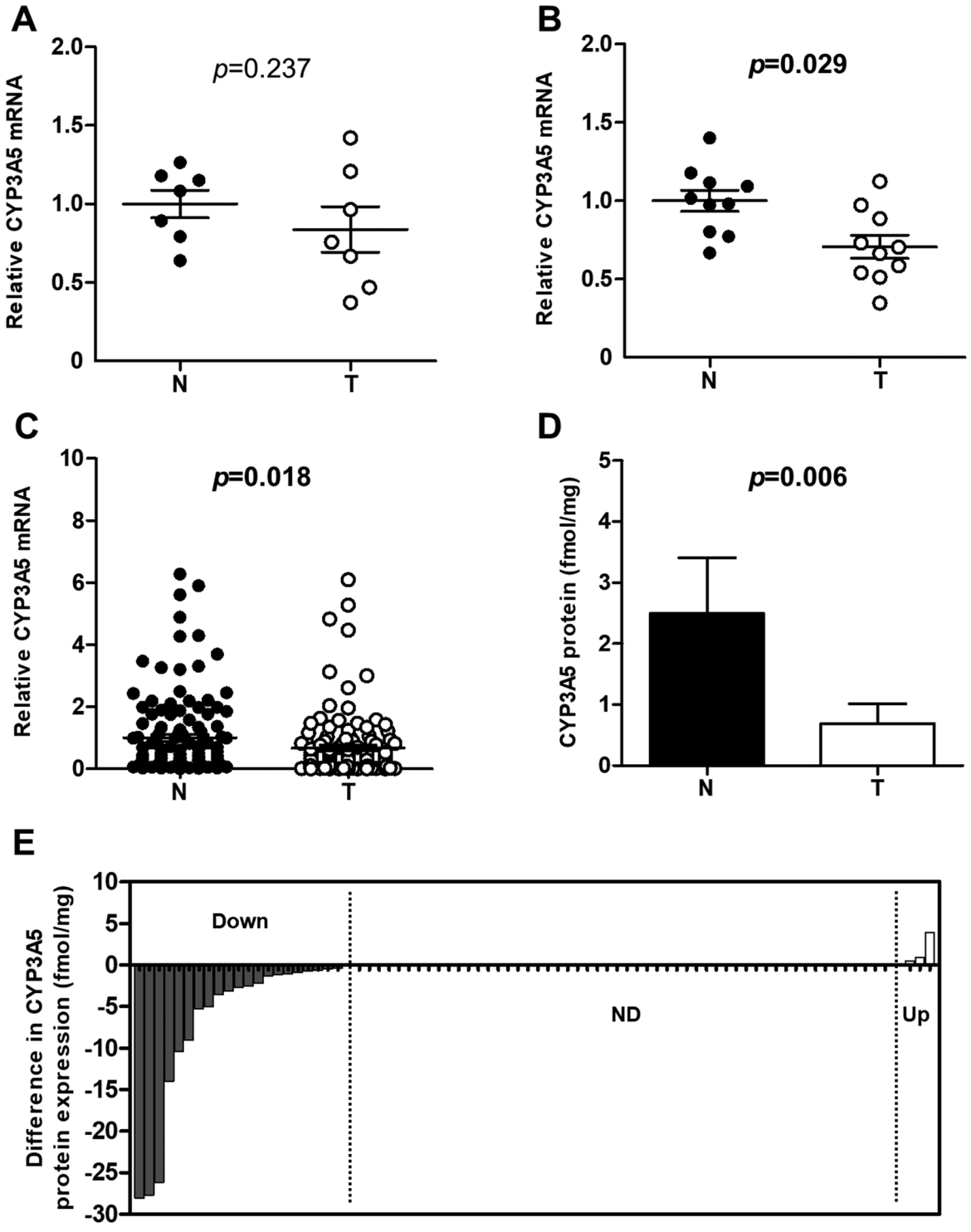

Figure 2. *Down-regulation of CYP3A5 expression levels in RCC tissues compared with that in the adjacent normal tissues. (A) and (B), difference in the level of CYP3A5 mRNA expression between adjacent normal tissues (N) and RCC tissues (T) in two datasets from the GEO database (A, GSE781; B, CSE6344). The expression level is presented as the ratio of the average value of CYP3A5 mRNA expression in the adjacent tissues. (C) and (D), differences in the expression levels of CYP3A5 mRNA (C, n=124) and protein (D, n=78) between adjacent normal tissues (N) and RCC tissues (T) in the cohort of this study. The expression levels are presented as mean±SE. (E), detailed expression changes of CYP3A5 protein from adjacent normal tissues to RCC tissues. The total protein fraction of the paired sample with RCC tissues and the adjacent normal tissues was 78. Down, Up, and ND indicate down-regulation, up-regulation, and not detected, respectively.*

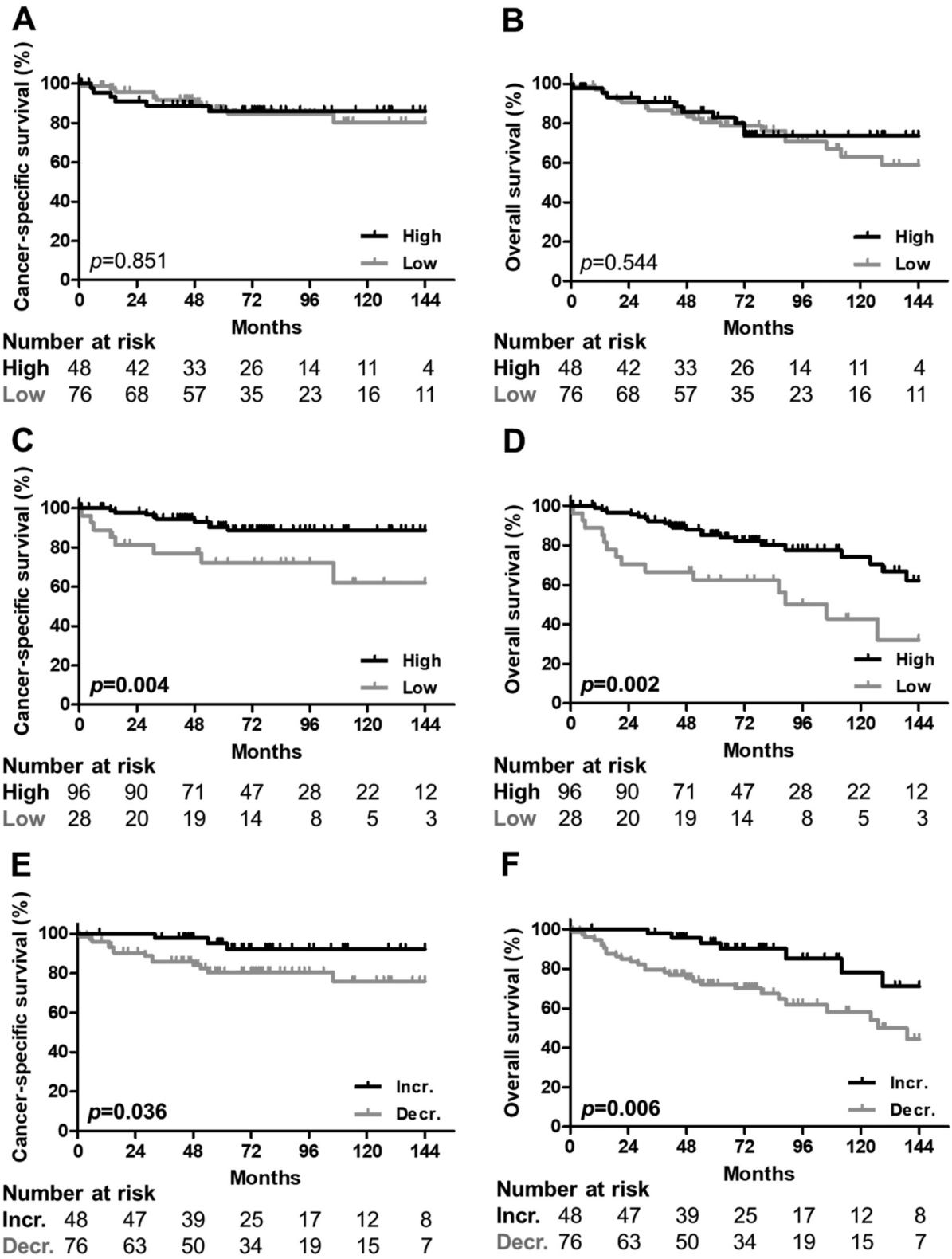

Figure 3. *Impact of CYP3A5 mRNA expression on the survival of RCC patients. (A)–(D), Kaplan–Meier analysis of CSS and OS based on the CYP3A5 mRNA expression in normal adjacent tissues (A and B) and RCC tissues (C and D). (E) and (F), Kaplan–Meier analysis of CSS (E) and OS (F) based on changes in the expression levels of CYP3A5 mRNA. Incr. and Decr. indicate increasing and decreasing, respectively.*

Table III. *Univariate and multivariate Cox analyses of cancer-specific survival and overall survival in 104 patients with RCC.*

CSS		Univariate			Multivariate		
Variable		Unadjusted OR	95% CI	*p*-Value	Adjusted OR	95% CI	*p*-Value
Age, years	≥65	0.809	0.381 to 1.717	0.580	-	-	-
Gender	Male	1.430	0.629 to 3.248	0.382	-	-	-
TNM stage	III+IV	4.750	1.748 to 10.130	**<0.001**	5.422	2.456 to 11.968	**<0.001**
Nuclear grade	3	2.159	0.971 to 4.802	0.073	-	-	-
Histological type	Clear cell	0.866	0.380 to 1.970	0.827	-	-	-
CYP3A5 mRNA expression	High	0.467	0.215 to 1.013	0.054	0.385	0.173 to 0.856	**0.019**
OS		Univariate			Multivariate		
Variable		Unadjusted OR	95% CI	*p*-Value	Adjusted OR	95% CI	*p*-Value
Age, years	≥65	1.173	0.642 to 2.142	0.604	-	-	-
Gender	Male	1.176	0.621 to 2.229	0.615	-	-	-
TNM stage	III+IV	3.229	1.748 to 5.963	**<0.001**	3.414	1.826 to 6.382	**<0.001**
Nuclear grade	3	1.501	0.754 to 2.989	0.264	-	-	-
Histological type	Clear cell	1.001	0.506 to 2.000	0.987	-	-	-
CYP3A5 mRNA expression	High	0.474	0.253 to 0.889	**0.002**	0.430	0.235 to 0.836	**0.012**

CSS, Cancer-specific survival; OS, overall survival; CYP, cytochrome P450; OR, odds ratio; CI, confidence interval. Bold values indicate statistical significance.

1.5-fold greater extent in the cortex than in the medulla of the human kidney (23). However, there is no information on the detailed position of malignant and adjacent normal tissues that were used in this study, which is possibly related to the disagreement between CYP3A5 protein expression and *CYP3A5*1* genotype in the kidney.

The difference in the level of CYP3A5 expression between normal tissues and cancer tissues has been determined in several types of cancer. The level of CYP3A5 expression in liver cancer and lung cancer tissues is lower than that in normal tissues (14, 15, 21). On the other hand, an increase in the level of CYP3A5 expression is observed in ovarian, colorectal, and breast cancers (27-29). A recent study has shown that there is no association between the *CYP3A5*3* genotype and risk of ovarian cancer (30), indicating that the level of CYP3A5 expression does not seem to affect some cancers, such as ovarian cancer. In RCC, the level of CYP3A5 expression was down-regulated in most cancer tissues as well as in liver and lung cancer tissues. Several RCC tissues did not express CYP3A5, whereas CYP3A5 expression was detected in the adjacent normal tissues. Given the position of malignant and adjacent normal tissues, the lower concordance rates of CYP3A5 protein expression with the *CYP3A5*1* genotype, especially in RCC tissues, may be partly explained by this down-regulation. A significant correlation between the expression levels of CYP3A5 mRNA and CYP3A5 protein indicates that the down-regulation of CYP3A5 protein seems to be transcriptionally regulated in RCC tissues. The transcriptional activity of CYP3A5 is possibly regulated by several nuclear receptors, such as pregnane X receptor (PXR; NR1I2), constitutive androstane receptor (CAR; NR1I3), hepatocyte nuclear factor 4α (HNF4α; NR2A1), and peroxisome proliferator-activated receptor alpha (PPARα; NR1C1), all of which are expressed in the kidney (31-34). These receptors activate the transcription of several targeted genes, including CYP3A5, after binding to ligands (35-37). Using datasets from the GEO database (GSE781 or CSE6344), the mRNA expression levels of three of these receptors, NR1I3, NR2A1, and NR1C1, in RCC tissues were significantly lower than those in the adjacent normal tissues ($p<0.001$ for NR1I3, $p=0.002$ for NR2A1, and $p=0.002$ for NR1C1; data not shown), indicating that the expression of these nuclear receptors might be involved in the regulation of CYP3A5 expression in RCC.

The effects of down-regulation of CYP3A5 on cancer cells were examined in liver and lung cancers, both *in vitro* and *in vivo*. In liver cancer, elevated CYP3A5 expression in cancer cells inhibits cell migration and invasion *via* ROS/mTORC2/p-AKT signaling (14). CYP3A5-inducd ROS accumulation inhibits AKT phosphorylation followed by a decrease in mTORC2 kinase activity, suggesting that CYP3A5 plays a protective role in cancer progression. In lung cancers, CYP3A5 represses the activation of Smad1 to inhibit lung cancer metastasis by interacting with ATOH8 which is a transcription factor that could increase the

phosphorylation of Smad1 (15). The interaction of CYP3A5 and ATOH8 results in a decrease in the Smad1 phosphorylation. The high CYP3A5 expression in these cancer tissues significantly increased the survival duration for these cancer patients, and CYP3A5 expression was identified as an independent prognostic factor in multivariate Cox analysis (14, 21). As our data were in accordance with these results, the high level of CYP3A5 mRNA expression significantly extended survival, and the level of CYP3A5 mRNA expression was identified as an independent prognostic factor for RCC patients. Indeed, the ROS/mTORC2/p-AKT signaling and Smad1 signaling pathways are reported to be important for RCC progression and for development of renal cells, respectively (38, 39). Although further investigation is needed, CYP3A5 might have a suppressive role in RCC by inducing ROS accumulation and decreasing Smad1 phosphorylation as well as in liver and lung cancers.

This study has two limitations, the number of events and the number of samples that expressed the CYP3A5 protein. Event numbers in RCC patients were low as the outcomes of RCC patients were relatively better than those of patients with other cancers, such as liver and lung cancers. Most of the patients included in this study were in relatively good condition because they could undergo surgery, which may also be related to the low number of events in this study. In addition, the number of patients who expressed the CYP3A5 protein was considerably lower than expected based on the allele frequency of *CYP3A5*1* in the Japanese population, especially in RCC tissues, due to the discordance of CYP3A5 protein expression with the *CYP3A5*1* genotype and down-regulation of CYP3A5 protein expression. Nonetheless, our results suggest that expression levels of CYP3A5 mRNA in RCC tissues significantly correlated with its protein expression and CYP3A5 protein expression is transcriptionally regulated in RCC tissues. Thus, CYP3A5 may be involved in RCC progression and may have a suppressive effect on RCC cells.

Conclusion

This study determined the detailed characteristics of CYP3A5 expression in RCC. The expression of CYP3A5 in RCC tissues was down-regulated compared to that in the adjacent normal tissues. The level of CYP3A5 mRNA expression in RCC correlated with several clinicopathological parameters and survival in RCC patients, suggesting that CYP3A5 may have a suppressive role in RCC. These findings provide basic information on CYP3A5 expression in the kidney, may facilitate better understanding of the importance of CYP3A5 in RCC, and may have implications for future prognostication and treatment planning for patients with RCC.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

J. Matsumoto: Conceptualization, methodology, data curation, writing - original draft, and funding acquisition. Y. Kotera: Formal analysis and data curation. K. Takeuchi: Formal analysis and data curation. S. Watari: Investigation and resources. H. Ueki: Investigation and resources. T. Koyama: Writing - review & editing and supervision. K. Wada: Writing - review & editing and supervision. M. Fujiyoshi: Writing - review & editing and supervision. Y. Nasu: Writing - review & editing and supervision. N. Ariyoshi: Writing - review & editing and supervision. All Authors have read and approved the final article.

Acknowledgements

The Authors acknowledge the members of the Department of Urology at Okayama University Hospital for their assistance in collecting patient information. They also thank the Central Research Laboratory, Okayama University Medical School for the provision of analytical instruments and Editage (ww.editage.jp) for English language editing.

This work was supported by the KAWASAKI Foundation for Medical Science and Medical Welfare and the JSPS KAKENHI (Grant-in-Aid for Young Scientists, Grant no. 20K16043).

References

1 Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, Heng DY, Larkin J and Ficarra V: Renal cell carcinoma. Nat Rev Dis Primers *3*: 17009, 2017. PMID: 28276433. DOI: 10.1038/nrdp.2017.9

2 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin *68(6)*: 394-424, 2018. PMID: 30207593. DOI: 10.3322/caac.21492

3 Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, Vakiti A, Rawla P and Barsouk A: Epidemiology of renal cell carcinoma. World J Oncol *11(3)*: 79-87, 2020. PMID: 32494314. DOI: 10.14740/wjon1279

4 Cancer Research UK: Office for National Statistics, Cancer survival by stage at diagnosis for England, 2019. Available at https://www.cancerresearchuk.org/about-cancer/kidney-cancer/survival [Last accessed on 21st March 2021]

5 Du Z, Chen W, Xia Q, Shi O and Chen Q: Trends and projections of kidney cancer incidence at the global and national levels, 1990-2030: a Bayesian age-period-cohort modeling study. Biomark Res *8*: 16, 2020. PMID: 32435498. DOI: 10.1186/s40364-020-00195-3

6 Daly AK: Significance of the minor cytochrome P450 3A isoforms. Clin Pharmacokinet *45(1)*: 13-31, 2006. PMID: 16430309. DOI: 10.2165/00003088-200645010-00002

7 Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K,

Boguski MS and Schuetz E: Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet *27(4)*: 383-391, 2001. PMID: 11279519. DOI: 10.1038/86882

8 Lin YS, Dowling AL, Quigley SD, Farin FM, Zhang J, Lamba J, Schuetz EG and Thummel KE: Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism. Mol Pharmacol *62(1)*: 162-172, 2002. PMID: 12065767. DOI: 10.1124/mol.62.1.162

9 Murray GI, McFadyen MC, Mitchell RT, Cheung YL, Kerr AC and Melvin WT: Cytochrome P450 CYP3A in human renal cell cancer. Br J Cancer *79(11-12)*: 1836-1842, 1999. PMID: 10206301. DOI: 10.1038/sj.bjc.6690292

10 Bolbrinker J, Seeberg S, Schostak M, Kempkensteffen C, Baelde H, de Heer E and Kreutz R: CYP3A5 genotype-phenotype analysis in the human kidney reveals a strong site-specific expression of CYP3A5 in the proximal tubule in carriers of the CYP3A5*1 allele. Drug Metab Dispos *40(4)*: 639-641, 2012. PMID: 22214815. DOI: 10.1124/dmd.111.042648

11 Fujita K: Cytochrome P450 and anticancer drugs. Curr Drug Metab *7(1)*: 23-37, 2006. PMID: 16454691. DOI: 10.2174/138920006774832587

12 Bertholee D, Maring JG and van Kuilenburg AB: Genotypes affecting the pharmacokinetics of anticancer drugs. Clin Pharmacokinet *56(4)*: 317-337, 2017. PMID: 27641154. DOI: 10.1007/s40262-016-0450-z

13 Lamba JK, Lin YS, Schuetz EG and Thummel KE: Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev *54(10)*: 1271-1294, 2002. PMID: 12406645. DOI: 10.1016/s0169-409x(02)00066-2

14 Jiang F, Chen L, Yang YC, Wang XM, Wang RY, Li L, Wen W, Chang YX, Chen CY, Tang J, Liu GM, Huang WT, Xu L and Wang HY: CYP3A5 functions as a tumor suppressor in hepatocellular carcinoma by regulating mTORC2/Akt signaling. Cancer Res *75(7)*: 1470-1481, 2015. PMID: 25649767. DOI: 10.1158/0008-5472.CAN-14-1589

15 Mao Q, Wang L, Liang Y, Dong G, Xia W, Hu J, Xu L and Jiang F: CYP3A5 suppresses metastasis of lung adenocarcinoma through ATOH8/Smad1 axis. Am J Cancer Res *10(10)*: 3194-3211, 2020. PMID: 33163265.

16 Japanese Urological Association, the Japanese Society of Pathology, and the Japan Radiological Society: General rule for clinical and pathological studies on renal cell carcinoma. 4th ed. Tokyo, Japan, Kanehara Publishers, 1999.

17 San SN, Matsumoto J, Saito Y, Koike M, Sakaue H, Kato Y, Fujiyoshi M, Ariyoshi N and Yamada H: Minor contribution of CYP3A5 to the metabolism of hepatitis C protease inhibitor paritaprevir *in vitro*. Xenobiotica *49(8)*: 935-944, 2019. PMID: 30227770. DOI: 10.1080/00498254.2018.1524947

18 El-Mokadem I, Fitzpatrick J, Bondad J, Rauchhaus P, Cunningham J, Pratt N, Fleming S and Nabi G: Chromosome 9p deletion in clear cell renal cell carcinoma predicts recurrence and survival following surgery. Br J Cancer *111(7)*: 1381-1390, 2014. PMID: 25137021. DOI: 10.1038/bjc.2014.420

19 Noll EM, Eisen C, Stenzinger A, Espinet E, Muckenhuber A, Klein C, Vogel V, Klaus B, Nadler W, Rösli C, Lutz C, Kulke M, Engelhardt J, Zickgraf FM, Espinosa O, Schlesner M, Jiang X, Kopp-Schneider A, Neuhaus P, Bahra M, Sinn BV, Eils R, Giese NA, Hackert T, Strobel O, Werner J, Büchler MW, Weichert W, Trumpp A and Sprick MR: CYP3A5 mediates basal and acquired therapy resistance in different subtypes of pancreatic ductal adenocarcinoma. Nat Med *22(3)*: 278-287, 2016. PMID: 26855150. DOI: 10.1038/nm.4038

20 Lolodi O, Wang YM, Wright WC and Chen T: Differential regulation of CYP3A4 and CYP3A5 and its implication in drug discovery. Curr Drug Metab *18(12)*: 1095-1105, 2017. PMID: 28558634. DOI: 10.2174/1389200218666170531112038

21 Qixing M, Juqing X, Yajing W, Gaochao D, Wenjie X, Run S, Anpeng W, Lin X, Feng J and Jun W: The expression levels of CYP3A4 and CYP3A5 serve as potential prognostic biomarkers in lung adenocarcinoma. Tumour Biol *39(4)*: 1010428 317698340, 2017. PMID: 28381170. DOI: 10.1177/1010428317698340

22 Schuetz EG, Schuetz JD, Grogan WM, Naray-Fejes-Toth A, Fejes-Toth G, Raucy J, Guzelian P, Gionela K and Watlington CO: Expression of cytochrome P450 3A in amphibian, rat, and human kidney. Arch Biochem Biophys *294(1)*: 206-214, 1992. PMID: 1550347. DOI: 10.1016/0003-9861(92)90159-t

23 Haehner BD, Gorski JC, Vandenbranden M, Wrighton SA, Janardan SK, Watkins PB and Hall SD: Bimodal distribution of renal cytochrome P450 3A activity in humans. Mol Pharmacol *50(1)*: 52-59, 1996. PMID: 8700118.

24 Fukuen S, Fukuda T, Maune H, Ikenaga Y, Yamamoto I, Inaba T and Azuma J: Novel detection assay by PCR-RFLP and frequency of the CYP3A5 SNPs, CYP3A5*3 and *6, in a Japanese population. Pharmacogenetics *12(4)*: 331-334, 2002. PMID: 12042671. DOI: 10.1097/00008571-200206000-00009

25 Hiratsuka M, Takekuma Y, Endo N, Narahara K, Hamdy SI, Kishikawa Y, Matsuura M, Agatsuma Y, Inoue T and Mizugaki M: Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population. Eur J Clin Pharmacol *58(6)*: 417-421, 2002. PMID: 12242601. DOI: 10.1007/s00228-002-0499-5

26 Zhou Y, Ingelman-Sundberg M and Lauschke VM: Worldwide distribution of cytochrome P450 alleles: a meta-analysis of population-scale sequencing projects. Clin Pharmacol Ther *102(4)*: 688-700, 2017. PMID: 28378927. DOI: 10.1002/cpt.690

27 Downie D, McFadyen MC, Rooney PH, Cruickshank ME, Parkin DE, Miller ID, Telfer C, Melvin WT and Murray GI: Profiling cytochrome P450 expression in ovarian cancer: identification of prognostic markers. Clin Cancer Res *11(20)*: 7369-7375, 2005. PMID: 16243809. DOI: 10.1158/1078-0432.CCR-05-0466

28 Kumarakulasingham M, Rooney PH, Dundas SR, Telfer C, Melvin WT, Curran S and Murray GI: Cytochrome p450 profile of colorectal cancer: identification of markers of prognosis. Clin Cancer Res *11(10)*: 3758-3765, 2005. PMID: 15897573. DOI: 10.1158/1078-0432.CCR-04-1848

29 Murray G, Patimalla S, Stewart K, Miller I and Heys S: Profiling the expression of cytochrome P450 in breast cancer. Histopathology *57(2)*: 202-211, 2021. PMID: 20716162. DOI: 10.1111/j.1365-2559.2010.03606.x

30 Fiszer-Maliszewska Ł, Łaczmański Ł, Dolińska A, Jagas M, Kołodziejska E, Jankowska M and Kuśnierczyk P: Polymorphisms of ABCB1, CYP3A4 and CYP3A5 genes in ovarian cancer and treatment response in poles. Anticancer Res *38(3)*: 1455-1459, 2018. PMID: 29491071. DOI: 10.21873/anticanres.12370

31 Miki Y, Suzuki T, Tazawa C, Blumberg B and Sasano H: Steroid and xenobiotic receptor (SXR), cytochrome P450 3A4 and multidrug resistance gene 1 in human adult and fetal tissues. Mol

Cell Endocrinol *231(1-2)*: 75-85, 2005. PMID: 15713537. DOI: 10.1016/j.mce.2004.12.005

32 Baes M, Gulick T, Choi HS, Martinoli MG, Simha D and Moore DD: A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. Mol Cell Biol *14(3)*: 1544-1552, 1994. PMID: 8114692. DOI: 10.1128/mcb.14.3.1544

33 Kritis AA, Argyrokastritis A, Moschonas NK, Power S, Katrakili N, Zannis VI, Cereghini S and Talianidis I: Isolation and characterization of a third isoform of human hepatocyte nuclear factor 4. Gene *173(2)*: 275-280, 1996. PMID: 8964514. DOI: 10.1016/0378-1119(96)00183-7

34 Sato K, Sugawara A, Kudo M, Uruno A, Ito S and Takeuchi K: Expression of peroxisome proliferator-activated receptor isoform proteins in the rat kidney. Hypertens Res *27(6)*: 417-425, 2004. PMID: 15253107. DOI: 10.1291/hypres.27.417

35 Burk O, Koch I, Raucy J, Hustert E, Eichelbaum M, Brockmöller J, Zanger UM and Wojnowski L: The induction of cytochrome P450 3A5 (CYP3A5) in the human liver and intestine is mediated by the xenobiotic sensors pregnane X receptor (PXR) and constitutively activated receptor (CAR). J Biol Chem *279(37)*: 38379-38385, 2004. PMID: 15252010. DOI: 10.1074/jbc.M404949200

36 Jover R, Bort R, Gómez-Lechón MJ and Castell JV: Cytochrome P450 regulation by hepatocyte nuclear factor 4 in human hepatocytes: a study using adenovirus-mediated antisense targeting. Hepatology *33(3)*: 668-675, 2001. PMID: 11230748. DOI: 10.1053/jhep.2001.22176

37 Janssen AW, Betzel B, Stoopen G, Berends FJ, Janssen IM, Peijnenburg AA and Kersten S: The impact of PPARα activation on whole genome gene expression in human precision cut liver slices. BMC Genomics *16*: 760, 2015. PMID: 26449539. DOI: 10.1186/s12864-015-1969-3

38 Xu M, Wang Y, Zhou LN, Xu LJ, Jin ZC, Yang DR, Chen MB and Zhu J: The therapeutic value of SC66 in human renal cell carcinoma cells. Cell Death Dis *11(5)*: 353, 2020. PMID: 32393791. DOI: 10.1038/s41419-020-2566-1

39 Ross MD, Martinka S, Mukherjee A, Sedor JR, Vinson C and Bruggeman LA: Math6 expression during kidney development and altered expression in a mouse model of glomerulosclerosis. Dev Dyn *235(11)*: 3102-3109, 2006. PMID: 16937370. DOI: 10.1002/dvdy.20934

Received March 22, 2021
Revised April 2, 2021
Accepted April 16, 2021