Differential Expression of Drug Uptake and Efflux Transporters in Japanese Patients with Hepatocellular Carcinoma

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Received July 9, 2014; accepted September 17, 2014

ABSTRACT

Targeted chemotherapy for hepatocellular carcinoma (HCC) is impaired by intrinsic and/or acquired drug resistance. Because drugs used in HCC therapy (e.g., anthracyclines or the tyrosine kinase inhibitor sorafenib) are substrates of uptake and/or efflux transporters, variable expression of these transporters at the plasma membrane of tumor cells may contribute to drug resistance and subsequent clinical response. In this study, the variability of expression of uptake transporters [organic cation transporter (OCT) 1 and OCT3] and efflux transporters [multidrug resistance 1 (MDR1)/P-glycoprotein, multidrug resistance protein (MRP) 1, MRP2, and breast cancer resistance protein (BCRP)], selected for their implication in transporting drugs used in HCC therapy, was investigated. HCC and corresponding nontumor tissue samples were collected from 24 Japanese patients at the time of surgery. Protein expression was determined by immunohistochemistry. Expression data were correlated with clinicopathological characteristics and patients’ outcome (median follow-up, 53 months). Generally, expression was highly variable among individual tumor samples. Yet median expression of OCT1, OCT3, and MDR1 in HCC was significantly lower (1.4-, 2.7-, and 2-fold, respectively) than in nontumor tissue, while expression of MRP2 persisted and BCRP showed a trend of increased levels in HCC. Patients with low BCRP expression had significantly shorter overall and recurrence-free survival times. Results suggest different expression patterns of drug transporters in HCC, which are associated only in part with clinicopathological characteristics. Detailed information on expression of drug transporters in HCC may be promising for individualization and optimization of drug therapy for liver cancer.

Introduction

Hepatocellular carcinoma (HCC) is the third-leading cause of cancer mortality worldwide (with its highest incidence in eastern Asia and the sub-Saharan region) despite recent improvement in early detection by imaging techniques as well as novel targeted therapy (El-Serag et al., 2008; Villanueva and Llovet, 2011; Forner et al., 2012; Llovet et al., 2012). Chronic hepatitis and cirrhosis, very often associated with viral infection by hepatitis B or hepatitis C, are the major underlying reasons for the development of HCC. Other risk factors are abnormal alcohol consumption, metabolic liver diseases (e.g., nonalcoholic steatohepatitis), and aflatoxin exposure. Irrespective of the etiology of HCC, only patients with early-stage HCC can benefit from radical therapies such as surgical resection, liver transplantation, or percutaneous local ablation. HCC patients diagnosed at intermediate to advanced stage are eligible for transarterial chemoembolization or sorafenib therapy when residual liver function and physiological status are preserved (Llovet and Bruix, 2008; Cheng et al., 2009).

Chemotherapy for HCC is impeded by the multidrug resistance (MDR) phenotype whereby tumor cells resistant to one anticancer drug are also resistant to drugs with completely different structures and modes of action (Baguley, 2010). Decreased drug uptake and increased drug efflux, mediated by integral membrane transporters, are considered important mechanisms of drug resistance (Gillett and Gottesman, 2010). Many different transporters are physiologically expressed in human hepatocytes, which take up endogenous substances and drugs across the sinusoidal membrane and efflux them into bile (Degorter et al., 2012; Chu et al., 2013). In the context of HCC therapy, the uptake transporters organic cation transporter (OCT) 1 (encoded by the SLC22A1 gene) and OCT3 (SLC22A3) as well as the ATP-binding cassette (ABC) efflux transporters MDR1/P-glycoprotein (ABCB1), multidrug resistance protein (MRP) 2 (ABCC2), and breast cancer resistance protein (BCRP; ABCC2) are of particular interest. They transport and confer resistance to anthracyclines, platinum drugs, and sorafenib (Cui et al., 1999; Burger et al., 2004; Yonezawa et al., 2006; Gillet and Gottesman, 2010; Herrera et al., 2013; Swift et al., 2013), which are commonly used in the treatment of HCC (El-Serag et al., 2008; Llovet et al., 2012). An increased expression of uptake transporters and a decreased expression of efflux transporters would favor the accumulation of cytostatic drugs.

This work was supported by the Wilhelm-Sander-Stiftung, München, Germany (Grant no.2010.059.1); by the Robert Bosch Stiftung, Stuttgart, Germany; and by the European Union [Grant FP7 PITN-GA-2009-238132].

dx.doi.org/10.1124/dmd.114.059832.

[This article has supplemental material available at dmd.aspetjournals.org.]

ABBREVIATIONS: ABC, ATP-binding cassette transporter; BCRP, breast cancer resistance protein; HCC, hepatocellular carcinoma; MDR, multidrug resistance; MRP, multidrug resistance protein; OCT, organic cation transporter; OS, overall survival; RFS, recurrence-free survival.
within the tumor cells. Thus, patient-specific expression of uptake and efflux drug transporters may contribute to the optimization of the selection of HCC drugs and/or adjustment of dosing.

Several studies have already investigated drug transporter expression in HCC, however, either with focus on a single transporter, e.g., BCRP (Sukowati et al., 2012) or MDR1 (Ng et al., 2000; Kato et al., 2001; Akimoto et al., 2006), or several ABC drug efflux (Nies et al., 2001; Zollner et al., 2005; Sun et al., 2010) or OCT drug uptake (Schaeffeler et al., 2011; Heise et al., 2012) transporters. An investigation comprising the simultaneous analysis of protein expression of these drug transporters has not yet been performed.

Therefore, this study aimed (i) to systematically investigate protein expression of OCT1, OCT3, MDR1, MRP2, and BCRP in HCC and corresponding nontumor samples from chemotherapy-naive patients with the same ethnic background and (ii) to evaluate drug transporter expression in association with clinicopathological characteristics and/or patient outcome.

### Materials and Methods

**Patients and Liver Tissues.** Formalin-fixed, paraffin-embedded samples of HCC and adjacent nontumor liver tissue were obtained from 24 Japanese patients who underwent partial liver resection performed in the Department of Surgery, Nara Medical University Hospital (Kashihara, Japan), and Department of Surgery, Kokuho Central Hospital (Shikigun, Japan) between January 2000 and June 2009. The study was approved by the local ethics committee, and all patients gave informed consent before surgery. HCC recurrence was assessed by ultrasound sonography, computed tomography, or magnetic resonance imaging every 2–3 months. One patient was excluded from analysis since he received presurgery chemotherapy (epirubicin). Seven patients received chemotherapy after surgery, including epirubicin (n = 4) and cisplatin as monotherapy (n = 1) or cisplatin in combination with 5-fluorouracil (n = 1) or with 5-fluorouracil and epirubicin (n = 1). None of the patients was treated with sorafenib. All HCC specimens were examined by an experienced pathologist, and histologic characterization was performed according to international criteria, i.e., grading (Hamilton and Aaltonen, 2000) and TNM stage (Singletary et al., 2003). Demographic and clinical data for the patients are summarized in Table 1.

**Antibodies.** Monoclonal antibodies were purchased that specifically detected the following ABC efflux transporters: JSB-1 against MDR1 (Scheffer et al., 2000) was from GeneTex, Inc. (Irvine, CA); QCRL-1 against MRP1 (Wright et al., 1998), M2III-6 against MRP2 (Nies et al., 2000), and BXP-21 against BCRP (Zen et al., 2007) were from Alexis Biochemicals (San Diego, CA). Generation and use of the polyclonal antibodies KEN and CGR specifically detecting human OCT1 and OCT3, respectively, have been described previously (Nies et al., 2009; Schaeffeler et al., 2011).

**Immunohistochemical Analysis.** Paraffin sections (3 μm) mounted on glass slides (DakoCytomation, Glostrup, Denmark) were deparaffinized with xylene (Merck KGaA, Darmstadt, Germany) and hydrated with 100, 96, and 70% ethanol. Antigen retrieval was performed in 10 mM citrate buffer, pH 6.0 (DakoCytomation), preheated to 99°C for 30 minutes. After blocking the endogenous peroxidase activity with peroxidase-blocking solution (DakoCytomation) for 15 minutes, the sections were incubated for 30 minutes at room temperature with primary antibodies against MDR1, MRP2, OCT1, and OCT3 or overnight at 4°C with primary antibodies against BCRP and MRP1. Primary antibodies were diluted in a commercially available diluent (DakoCytomation) as necessary.

**Immunohistochemical Analysis.** Paraffin sections (3 μm) mounted on glass slides (DakoCytomation, Glostrup, Denmark) were deparaffinized with xylene (Merck KGaA, Darmstadt, Germany) and hydrated with 100, 96, and 70% ethanol. Antigen retrieval was performed in 10 mM citrate buffer, pH 6.0 (DakoCytomation), preheated to 99°C for 30 minutes. After blocking the endogenous peroxidase activity with peroxidase-blocking solution (DakoCytomation) for 15 minutes, the sections were incubated for 30 minutes at room temperature with primary antibodies against MDR1, MRP2, OCT1, and OCT3 or overnight at 4°C with primary antibodies against BCRP and MRP1. Primary antibodies were diluted in a commercially available diluent (DakoCytomation) as necessary.
The sections were incubated for 30 minutes at room temperature with goat secondary antibodies against mouse and rabbit IgG conjugated to peroxidase-labeled polymer (EnVision+ System; DakoCytomation). The antigen-antibody immunoreaction was visualized with 3,3′-diaminobenzidine tetrahydrochloride used as chromogen, followed by counterstaining with Mayer’s hematoxylin. The slides were then dehydrated in alcohol and xylene and mounted with several drops of permanent aqueous mounting medium.

**Evaluation of Immunostaining.** Immunohistochemical staining was evaluated by two independent observers with no knowledge of patient characteristics. Discrepancies were resolved by consensus. Intensity of membrane staining of tumor cells was assessed and scored as 0 (no staining), 1 (weak), 2 (medium), and 3 (strong) as described previously (Nies et al., 2001; Schaeffeler et al., 2011). Examples of different staining intensities are given in Supplemental Fig. 1. By multiplying the intensity score and the proportion of immunopositive cells (0–100%), a semiquantitative staining score, ranging from 0 to 300, was calculated as previously described (Schaeffeler et al., 2011).

**Statistical Analysis.** Data were analyzed using Prism software version 5.04 (GraphPad Software, Inc., La Jolla, CA). Data are expressed as medians and ranges. The Mann-Whitney test or the Kruskal-Wallis test was used as appropriate to calculate the effect of clinicopathological parameters (age, sex, tumor stage, tumor grade, and tumor size) on drug transporter expression. Additionally, multivariate linear regression models were calculated with statistics software R-3.1.0 (http://www.r-project.org). To meet the Gaussian assumption, expression data were log-transformed.

**TABLE 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OCT1</th>
<th>OCT3</th>
<th>BCRP</th>
<th>MDR1</th>
<th>MRP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at surgery</td>
<td>0.169</td>
<td>0.216</td>
<td>0.581</td>
<td>0.747</td>
<td>0.314</td>
</tr>
<tr>
<td>Sex</td>
<td>0.368</td>
<td>0.911</td>
<td>0.084</td>
<td>0.652</td>
<td>0.601</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>0.220</td>
<td>0.0095</td>
<td>0.119</td>
<td>0.711</td>
<td>0.057</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>0.060</td>
<td>0.897</td>
<td>0.059</td>
<td>0.068</td>
<td>0.948</td>
</tr>
<tr>
<td>Tumor size</td>
<td>0.597</td>
<td>0.025</td>
<td>0.038</td>
<td>0.459</td>
<td>0.648</td>
</tr>
</tbody>
</table>

P values were determined using Mann-Whitney or Kruskal-Wallis test as appropriate.
To analyze the impact of drug transporter expression on overall survival (OS) and recurrence-free survival (RFS), patients who showed expression levels below the median were classified as “low expressers,” versus “high expressers” for those with expression levels at or above the median. To analyze the effect of clinicopathological parameters on OS and RFS, patients were grouped according to the criteria given in Table 1. Kaplan-Meier curves were analyzed by

**Fig. 3.** Association of drug transporter expression with tumor characteristics. Univariate analysis of drug transporter expression with tumor stage (A), tumor grade (B), and tumor size (C). OCT3 expression was significantly associated with tumor stage and tumor size and BCRP expression with tumor size. Sample sizes were as follows: pT1, 9; pT2, 8; pT3, 6; G1, 15; G2, 8; size < 20 mm, 6; size ≥ 20 mm, 17. *P < 0.05; **P < 0.01.
log-rank Mantel-Cox tests. To account for a combination of expression of different transporters, Cox proportional hazard models were calculated with the survival plug-in for R (Fox and Carvalho, 2012). All tests were two-sided, and $P < 0.05$ was considered to be significant.

**Results**

**Protein Expression Analysis of Drug Uptake Transporters**

OCT1 and OCT3 and Drug Efflux Transporters BCRP, MDR1, MRP2, and MRP1 in HCC. To systematically evaluate expression of drug transporters in HCC tissues, expression levels of OCT1, OCT3, BCRP, MDR1, MRP2, and MRP1 were analyzed by semiquantitative immunohistochemistry as previously described (Schaeffeler et al., 2011). In 91.3% of cases (21/23), HCC samples showed lower OCT1 staining intensity (median, 140; range, 0–300) compared with the corresponding nontumor tissues (median, 200; range, 100–300; Fig. 1A), resulting in a 1.4-fold difference. As expected, OCT1 was localized at the basolateral membrane of hepatocytes in the nontumor tissue (Fig. 1B) as well as of tumor cells, regardless of whether HCC had a trabecular (Fig. 1C) or pseudoglandular (Fig. 1D) growth pattern. Similar to OCT1, OCT3 expression was significantly reduced, by a factor of 2.7, in HCC (median, 60; range, 10–250) compared with nontumor tissue (median, 160; range, 80–200; Fig. 1E). Distinct basolateral membrane staining was detectable in hepatocytes in the nontumor tissue (Fig. 1F) and also in tumor cells of HCC growing in a trabecular (Fig. 1G) or pseudoglandular (Fig. 1H) pattern.

The BCRP immunostaining score was higher in HCC than in matched nontumor tissue in 73.9% of cases (17/23), leading to a 1.6-fold-higher median BCRP staining score in HCC (median, 160; range, 20–270; Fig. 2A). BCRP was localized at the apical membrane of hepatocytes (Fig. 2B). In HCC, BCRP was also localized at the plasma membrane of tumor cells and staining appeared to be more intense on the apical site of the cells arranged in trabecular structures (Fig. 2C) and the luminal surfaces of those with pseudoglandular arrangements (Fig. 2D). The median staining score of MDR1 was significantly decreased, by a factor of 2, in HCC (median, 50; range, 0–270) compared with nontumor samples (median, 100; range, 50–200; Fig. 2E). Positive MDR1 immunostaining was found exclusively in the apical membrane of hepatocytes (Fig. 2F). In trabecular HCC (Fig. 2G) and in pseudoglandular HCC (Fig. 2H), MDR1 staining was found almost exclusively on the apical site of tumor cells. Median MRP2 staining intensity was not significantly different between nontumor and HCC samples ($P = 0.513$; Fig. 2I). Similar to localization of BCRP and MDR1, MRP2 was localized in the apical membrane of hepatocytes (Fig. 2J) as well as in the apical membrane of tumor cells that were arranged either in a trabecular pattern (Fig. 2K) or in a pseudoglandular pattern (Fig. 2L). In line with previous studies (Nies et al., 2001), immunohistochemical analysis revealed only occasional intracellular MRP1 staining in a few nontumor and HCC cells (Supplemental Fig. 2) and was therefore not quantified.

**Correlation of Drug Transporter Expression with Clinicopathological Characteristics.** Correlations of transporter immunoreactivities and clinicopathological characteristics were assessed first by univariate analyses. Transporter immunoreactivities were not associated with age at surgery or sex (Table 2). However, significant associations were observed for OCT3 and BCRP but not for OCT1, MDR1, and MRP2 (Table 2). In particular, a tumor size of ≥20 mm was associated with decreased OCT3 and BCRP expression, and additionally, OCT3 expression was lower in higher tumor stage (Fig. 3). Moderate associations were observed between tumor grade and expression of BCRP, MDR1 (higher in G2), and OCT1 (lower in G2). By multivariate analyses, OCT3 and BCRP expression remained significantly associated with tumor size (Supplemental Table 1). Additionally, significant relationships of OCT3 and BCRP expression with age and sex, respectively, were observed.

**Relationship between Drug Transporter Expression or Clinicopathological Characteristics and Patient Survival.** Associations between drug transporter expression as well as clinicopathological characteristics and patient survival were assessed by calculating Kaplan-Meier survival estimates (Fig. 4; Table 3). Median follow-up of the whole study population was 1604 days (range, 23–3331). BCRP expression was significantly correlated with OS and RFS (Fig. 4; Table 3). Patients with low and high BCRP expression showed a median OS of 1517 days (range, 23–1638 days) and 2847 days (range, 1436–3331 days), respectively. RFS was 654 days (range, 23–1515 days) in BCRP low expressers versus 1684 days (range, 561–2152) in high expressers. Because some of the patients received chemotherapy after surgery ($n = 7$), we reanalyzed BCRP expression versus clinical outcome only in patients without chemotherapy, again resulting in a significant correlation between high BCRP expression and better OS as well as RFS (Supplemental Table 2).

In contrast, the expression of the uptake transporters OCT1 and OCT3 did not influence patient outcome, neither individually (Table 3) nor in combination with each other (Table 4) or with MDR1 (Table 5).

**Discussion**

Resistance to drug therapy remains a major challenge in HCC treatment despite successful advances using targeted therapies (Llovet and Bruix, 2008; Cheng et al., 2009). Because the organic cation uptake transporters OCT1 and OCT3 (Yonezawa et al., 2006; Herraez et al., 2013; Swift et al., 2013) and the ABC efflux transporters

![Fig. 4. Association of BCRP expression with survival (A) and RFS time (B). Patients were stratified into two groups having BCRP expression levels either below the median (low BCRP expression; $n = 11$) or at or above the median (high BCRP expression; $n = 12$). Kaplan-Meier curves were generated and analyzed using log-rank Mantel-Cox tests. Patients with low BCRP protein expression had a worse survival ($P = 0.007$) and a shorter recurrence-free time ($P = 0.002$).](image-url)
MDR1, MRP2, and BCRP (Cui et al., 1999; Burger et al., 2004; Gillet and Gottesman, 2010) transport drugs that are used in HCC therapy, protein expression of these transporters in HCC may be of interest and have consequences for clinical outcome. For instance, doxorubicin and platinum compounds are used for transarterial chemoembolization treatment of intermediate-stage HCC and the tyrosine kinase inhibitor sorafenib for systemic treatment of advanced-stage HCC (Llovet et al., 2012). While other studies have already investigated expression of either single transporters in HCC [e.g., BCRP (Sukowati et al., 2012) and MDR1 (Ng et al., 2000; Kato et al., 2001; Akimoto et al., 2006)] or only the ABC or the OCT drug transporters (Nies et al., 2001; Zollner et al., 2005; Sun et al., 2010; Schaeffeler et al., 2011; Heise et al., 2012), this study is the first to analyze simultaneously the correlation of expression of six efflux and uptake transporters to clinicopathological characteristics and clinical outcome. Although immunohistochemistry used to determine protein expression is based on semiquantitative scoring with some limitations, this technique is commonly used in clinical tumor diagnostics (e.g., estrogen receptor, Ki-67) due to a simple and cost-saving application.

Intrinsic resistance of various tumor entities to anticancer drugs already at onset of therapy has been linked to low expression of drug uptake transporters and/or high expression of drug efflux transporters in tumor tissue, thus indicating that the expression of those transporters is an important contributor to drug response (Gillet and Gottesman, 2010). Therefore, we investigated the expression of the clinically relevant uptake transporters OCT1 and OCT3 and the efflux transporters MDR1, BCRP, and MRP2 in HCC in comparison with the corresponding non-tumor tissue. Of note, all of our HCC samples were derived from chemotherapy-naive patients to exclude confounding of transporter protein expression by regulation (e.g., inhibition or induction) via chemotherapeutic agents such as anthracyclines and platinum compounds. Previous data have shown that indeed both drug classes may alter membrane transporter expression in cancer cell lines and in clinical samples (Sun et al., 2010; Herraez et al., 2012; Sukowati et al., 2012).

Our finding that OCT1 protein levels are significantly lower in HCC tissue than in nontumor tissue from Japanese patients—mainly with hepatitis C etiology—is in line with previous reports on Caucasian HCC patients of various etiologies (Schaeffeler et al., 2011; Heise et al., 2012). This indicates that OCT1 downregulation appears to be a general feature of HCC irrespective of etiology or ethnicity. OCT3 protein expression was lower in HCC than in nontumor tissue as well. To our knowledge, this is the first study systematically reporting on OCT3 protein levels in HCC tissue. Our previous observation that OCT3 protein expression is not decreased in a limited number of samples from Caucasian HCC patients (Schaeffeler et al., 2011) may be because OCT3 expression—in contrast to OCT1 expression—is affected by etiology. Distinct gene expression patterns have been described for hepatitis B and hepatitis C positive HCC (Okabe et al., 2001). With regard to the drug efflux transporters BCRP, MDR1, and MRP2, their expression in HCC tissue in comparison with nontumor tissue did not follow a uniform trend, with upregulation observed in some and down-regulation in others. Median expression in HCC compared with nontumor tissue was lower (MDR1), higher (BCRP), or not changed (MRP2). This is in line with other reports (Ng et al., 2000; Nies et al., 2001; Zollner et al., 2005; Sun et al., 2010; Sukowati et al., 2012) and most likely reflects the molecular heterogeneity of HCCs (Zollner et al., 2005; Thorgerisson et al., 2006).

Next, we attempted to identify whether clinico-pathological characteristics may predict transporter expression in HCC tissue. Despite the small number of samples and the well known molecular heterogeneity of HCC (Zollner et al., 2005; Thorgerisson et al., 2006), our univariate and multivariate analyses showed that larger tumors are apparently associated with lower OCT3 and BCRP protein levels. Because sorafenib, which is used for systemic treatment of advanced-stage HCC (Llovet et al., 2012), is effluxed by BCRP (Burger et al., 2004), we speculate that patients with larger tumors and hence decreased BCRP expression may benefit from treatment with sorafenib.

Finally, we observed that patients with high BCRP levels had better outcomes, taking into account that our study population was small and results were based on semiquantitative immunohistochemical scoring. One function of BCRP is to protect cells against accumulation of harmful substances by pumping out environmental or endogenous toxicants (Huls et al., 2009). In the case of high BCRP expression/function, we suggest a protective role of BCRP for cells because BCRP actively effluxes dietary carcinogens such as the hepatocarcinogen aflatoxin B1 or heterocyclic amines (van Herwaarden and Schinkel, 2006). Moreover, BCRP has been shown to minimize

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### Table 3

Univariate correlation analysis of drug transporter expression and clinicopathological parameters with OS and RFS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OS</th>
<th></th>
<th>RFS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>HR</td>
<td>95% CI</td>
<td>P</td>
<td>HR</td>
</tr>
<tr>
<td>BCRP expression</td>
<td>0.007</td>
<td>11.61</td>
<td>1.93–69.73</td>
<td>0.002</td>
</tr>
<tr>
<td>MDR1 expression</td>
<td>0.868</td>
<td>0.86</td>
<td>0.15–5.05</td>
<td>0.355</td>
</tr>
<tr>
<td>MRP2 expression</td>
<td>0.902</td>
<td>0.90</td>
<td>0.18–4.65</td>
<td>0.496</td>
</tr>
<tr>
<td>OCT1 expression</td>
<td>0.296</td>
<td>2.27</td>
<td>0.49–10.59</td>
<td>0.348</td>
</tr>
<tr>
<td>OCT3 expression</td>
<td>0.322</td>
<td>0.49</td>
<td>0.09–2.52</td>
<td>0.722</td>
</tr>
<tr>
<td>Age at surgery</td>
<td>0.362</td>
<td>0.31</td>
<td>0.03–3.82</td>
<td>0.752</td>
</tr>
<tr>
<td>Sex</td>
<td>0.573</td>
<td>1.72</td>
<td>0.26–11.35</td>
<td>0.236</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>0.398</td>
<td></td>
<td></td>
<td>0.648</td>
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<tr>
<td>Tumor grade</td>
<td>0.561</td>
<td>0.63</td>
<td>0.13–3.01</td>
<td>0.149</td>
</tr>
<tr>
<td>Tumor size</td>
<td>0.367</td>
<td>0.47</td>
<td>0.09–2.41</td>
<td>0.881</td>
</tr>
<tr>
<td>Chemotherapy after surgery</td>
<td>0.370</td>
<td>0.48</td>
<td>0.09–2.42</td>
<td>0.265</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio.

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### Table 4

Multivariate correlation analysis of OCT1 and OCT3 expression with OS and RFS

<table>
<thead>
<tr>
<th>Expression of</th>
<th>OS</th>
<th></th>
<th>RFS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>HR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>OCT1</td>
<td>0.393</td>
<td>2.28</td>
<td>0.49–10.52</td>
<td>0.570</td>
</tr>
<tr>
<td>OCT3</td>
<td>0.45</td>
<td>0.07–2.68</td>
<td>0.76</td>
<td>0.76</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio.
cellular accumulation of porphyrins, including heme (Krishnamurthy et al., 2004). The regulation of intracellular porphin levels is important because excess levels may ultimately lead to generation of cell-damaging reactive oxygen species and to the collapse of mitochondrial function (Krishnamurthy et al., 2004). A protective role of BCRP for cells is supported by a study on intrahepatic cholangiocarcinoma—a subtype of liver cancer—in which better prognosis, similarly to the results of our study, was associated with high BCRP protein expression levels (Larbcharoensub et al., 2011). Mechanistically it is still unclear which underlying factors definitively contribute to better prognosis. However, it has been recently shown that hepatic cancer stem cells are responsible for tumor relapse, metastasis, and chemoresistance (Yamashita and Wang, 2013). Indeed, immuno-histochemical studies of stem cell markers suggest that HCCs are histologically heterogeneous and contain a subset of cells expressing a variety of stem cell markers (e.g., Ma et al., 2007). Regarding intrahepatic cholangiocarcinoma, it has been suggested that loss of BCRP expression renders progenitor cells prone to carcinogenesis and worsens the prognosis (Larbcharoensub et al., 2011). Thus, we suggest similar mechanisms to explain our association of better outcome and high BCRP expression.

Our study has some limitations. First, the study design was retrospective. Second, the number of HCC samples was small, and a larger cohort is warranted to confirm our data. Finally, we did not investigate whether genetic variation in the selected transporters may contribute to transporter expression in HCC because the number of samples was limited.

In summary, we investigated the expression of membrane transporters relevant for drugs used in HCC therapy. In our study including a limited number of HCC patients with Asian ethnic background, we showed that the expression of selected uptake and efflux transporters (particularly BCRP) is highly variable. BCRP expression may be a predictor for patient outcome in HCC. To this end, our work highlights the need for comprehensive studies on transporter expression in cancer, including interindividual variability and potential consequences for drug therapy.

Acknowledgments

The authors thank Dr. Salio Koh (Department of Surgery, Nara Medical Center, Nara, Japan), Dr. Takashi Yoshikawa and Dr. Yukishige Yamada (Department of Surgery, Kooko Central Hospital, Shikagun, Japan), Dr. Daisuke Hokuto (Department of Surgery, Nara Medical University, Kashihara, Japan), and Noboru Konishi (Department of Pathology, Nara Medical University) for providing liver tissue samples and Silvia Hubner (Stuttgart, Germany) for excellent technical assistance.

Authorship Contributions

Participated in research design: Namisaki, Schaefeller, Schwab, Nies
Conducted experiments: Namisaki, Schaefeller, Nies
Performed data analysis: Namisaki, Schaefeller, Fukui, Yoshiji, Nakajima, Fritz, Nies.

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proteins MRP1, MRP2, MRP3, MRP5, and MDR3 P-glycoprotein with a panel of monoclonal antibodies. *Cancer Res* **60**:5269–5277.


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