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Significance of OCT3/SLC22A3, Organic Cation Transporter 3, Expression for the Cytotoxic Effect of Oxaliplatin in Colorectal Cancer

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Running title: Relation between OCT3 expression and oxaliplatin efficacy

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Abstract

The effect of oxaliplatin against colorectal cancer is superior to that of cisplatin, but the molecular mechanism(s) involved is not clear. We previously found that oxaliplatin, but not cisplatin, was transported by human (h) and rat OCT3/SLC22A3, organic cation transporter 3. In the present study, we examined whether hOCT3 was significantly involved in the oxaliplatin-induced cytotoxicity and accumulation of platinum in colorectal cancer. The level of hOCT3 mRNA in the colon was 9.7-fold higher in cancerous than normal tissues in six Japanese patients (P=0.0247). In human colorectal cancer-derived cell lines, the mRNA of hOCT3 was highly expressed compared to that of other organic cation transporters. The release of LDH and accumulation of platinum on oxaliplatin treatment were increased in SW480 cells transfected with hOCT3 cDNA compared to empty vector-transfected cells. T84 and SW837 cells, with high levels of hOCT3, released more LDH and accumulated more platinum after oxaliplatin treatment than low hOCT3-expressing cells such as SW480, HCT116, HT29 and Lovo. However, the amount of platinum accumulated following cisplatin treatment did not differ among these six cells. The levels of hOCT3 expression in colon and rectum were also higher in cancerous than normal tissues in Caucasian patients as determined by dot blotting. In conclusion, the hOCT3-mediated uptake of oxaliplatin into the cancers was suggested to be important for its cytotoxicity, and hOCT3 expression may be a marker for cancer chemotherapy including oxaliplatin.

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Introduction

Platinum agents are widely used in the treatment of cancers. Cis-diamminedichloroplatinum II (cisplatin) was the first platinum agent to be synthesized, and has played an essential role in cancer chemotherapy for 30 years. However, severe nephrotoxicity and an increase in resistance to cisplatin-therapy limit continuous clinical use. Trans-L-1,2-diaminocyclohexaneoxalatoplatinum II (oxaliplatin) is a third-generation platinum agent, which is less nephrotoxic than cisplatin. Moreover, its spectrum of activity and mechanisms of action or resistance differ from those of cisplatin (Raymond et al., 2002; Fuertes et al., 2003; Wang and Lippard, 2005). Oxaliplatin is used against colorectal cancers as a key drug of FOLFOX regimens, and its objective response rate for colorectal cancer is superior to cisplatin (Loehrer et al., 1988; Grem et al., 1993; de Gramont et al., 2000; Andre et al., 2004). However, the molecular mechanism(s) underlying the clinical results, and why oxaliplatin has such a potent anti-colorectal cancer effect compared to cisplatin, is unclear.

The organic cation transporters (OCT/SLC22A) and multidrug and toxin extrusion (MATE/SLC47A) family transport cationic drugs, toxins, and endogenous metabolites (Inui et al., 2000; Terada and Inui, 2008). Previously, we elucidated that the severity of the nephrotoxicity of platinum agents depends on the amount of platinum accumulated in the kidney, and OCT and MATE could play the predominant role in the renal handling of these agents (Yonezawa et al., 2005; Yonezawa et al., 2006; Yokoo et al., 2007). We also reported that oxaliplatin, but not cisplatin, was selectively transported by rat (r) or human (h) organic cation transporter 3 (OCT3/SLC22A3) (Yonezawa et al., 2006; Yokoo et al., 2007). OCT3 mRNA was found in the placenta, intestine, heart, brain, and kidney, but the distribution in the membrane and physiological role of OCT3 are not yet clearly understood (Kekuda et al., 1998).

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We hypothesized that the substrate specificity and expression level of OCT3 affect the difference in the anticancer effect of platinum agents against colorectal cancer. In the present study, we examined and compared the level of OCT3 mRNA in colorectal cancer and normal colorectum, and between colorectal cancer-derived cell lines. In addition, the cytotoxicity and platinum accumulation in cultured cells caused by the treatment with oxaliplatin or cisplatin were examined.

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Materials and Methods

Cell culture

Human colorectal cancer-derived cell lines, T84 (CCL-248; American Type Culture Collection, Manassas, VA), SW480 (CCL-228; American Type Culture Collection), HCT116 (91091005; European Collection of Cell Cultures, Wiltshire, UK), HT29 (HTB-38; American Type Culture Collection), SW837 (JCRB9115; Health Science Research Resources Bank, Osaka, Japan), and Lovo (JCRB9083; Health Science Research Resources Bank) were used. HEK293 cells (CRL-1573; American Type Culture Collection) were used as a host for gene transfection (Yonezawa et al., 2006). Cell lines were cultured in an atmosphere of 5% CO_2-95% air at 37°C. Dulbecco's modified Eagle's medium (DMEM) (Sigma Chemical Co., St. Louis, MO) with 10% fetal bovine serum (FBS) (Invitrogen Life Technology Co., Carlsbad, CA) was used for SW480, HT29, HCT116, Lovo, and HEK293. DMEM containing 10% FBS and 1% non-essential amino acid (Invitrogen) was used for SW837, and a 1:1 mixture of DMEM and nutrient mixture F12 Ham (Sigma) with 10% FBS was used for T84. T84, HT29, and Lovo were seeded onto 24-well plates or 96-well plates at a density of 4.0×10^5 cells/ml. SW480 and HCT116 were seeded at 2.0×10^5 cells/ml, and SW837 was at 5.0×10^5 cells/ml. Seventy-two hours after the seeding, the cells were used for the experiments.

Transfection

For a transient expression system, pCMV6-XL4 plasmid vector DNA (OriGene Technologies, Rockville, MD) containing hOCT3 cDNA was purified using an EndoFree Plasmid Mega Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions (Yonezawa et al., 2006). The day before transfection, HEK293 or SW480 cells were seeded onto poly-D-lysine-coated and non-coated 24-well plates, respectively. The cells were

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transfected with 800 ng of plasmid DNA per well in a combination of empty vector and hOCT3 cDNA using 2 μl of Lipofectamine 2000 (Invitrogen) per well according to the manufacturer's instructions. The amount of hOCT3 cDNA was 800 ng except in the experiment examining the transporter cDNA-dependence. Forty-eight hours after the transfection, the cells were used for the experiments.

Uptake experiment

Cellular uptake of [^{3}H]1-methyl-4-phenylpyridium acetate (MPP) (2.7 TBq/mmol, PerkinElmer Life Analytical Science, Boston, MA) was measured with monolayer cultures grown on 24-well plates. The composition of the incubation buffer was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, and 5 mM HEPES (pH 7.4 adjusted with NaOH). As previously reported, experiments on the uptake were performed (Urakami et al., 2004).

For measurement of the cellular accumulation of oxaliplatin or cisplatin, cells seeded on 24-well plates were incubated with DMEM containing 10% FBS and oxaliplatin (Wako Pure Chemical Industries, Osaka, Japan) or cisplatin (Sigma) for 2 min or 1 h. After this incubation, the monolayers were rapidly washed twice with ice-cold incubation buffer containing 3% bovine serum albumin (Nacalai Tesque, Kyoto, Japan) and then washed three times with ice-cold incubation buffer. The cells were solubilized in 0.5 N NaOH, and the amount of platinum was determined using inductively coupled plasma-mass spectrometry (ICP-MS) by the Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd. (Wakayama, Japan). The protein content of the cell monolayers solubilized in 0.5 N NaOH was determined with a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA).

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Cytotoxicity assay

The cytotoxicity of oxaliplatin was measured with cells seeded on 24-well plates for the lactate dehydrogenase (LDH) assay, and on 96-well plates for the caspase 3/7 assay. Cells were incubated with medium containing oxaliplatin for 6 h for the LDH assay. After removal of the medium, a drug-free medium was added to the wells. After incubation for 24 h, the medium was collected, and the LDH activity in it was measured using a LDH Cytotoxicity Detection Kit (Takara Bio Inc., Shiga, Japan), according to the manufacturer's instructions. LDH release (%) was calculated as described previously (Yonezawa et al., 2006). For the caspase assay, cells were incubated with medium containing oxaliplatin for 8 h. After the incubation, caspase 3/7 activity was determined by using a Caspase-Glo 3/7 Assay (Promega, Madison, WI), according to the manufacturer's instructions. Caspase activity (fold increase) represents (caspase 3/7 activity in oxaliplatin-treated cells)/(caspase 3/7 activity in cells without oxaliplatin).

Isolation of total RNA and real-time PCR

Total RNA was isolated from each cell line on 24-well plates using an RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions, and the concentrations of total RNA were measured by spectrophotometry. Total RNA was reverse-transcribed with random hexamers using Superscript II reverse-transcriptase (Invitrogen), followed by digestion with RNase H (Invitrogen). For the detection of the expression of hOCT3 mRNA in cancerous or normal colon and rectum, the same batch of cDNA samples as used by Terada et al. (Terada et al., 2005) were subjected to real-time PCR. Detailed information about the patients was given in that report (Terada et al., 2005). The conditions and primer-probe sets for real-time PCR were described previously (Motohashi et al., 2002; Masuda et al., 2006). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA level was used as an internal

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control. This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee.

Cancer profiling array

The cDNA cancer profiling array, Cancer Profiling Array I, from Clontech Laboratories, Inc. (Mountain View, CA) was used. It includes normalized cDNAs from cancer and corresponding normal tissues from individual patients, amplified using SMART™ technology. Preparation of the cDNA probe for hOCT3, hybridization to the array and the signal detection on X-ray film were carried out using the DIG High Prime DNA Labelling and Detection Starter Kit II (Roche Ltd., Basel, Switzerland) according to the manufacturer's instructions. The relative intensity of each dot was determined densitometrically using ImageJ 1.38x (National Institutes of Health, Bethesda, MD).

Statistical analysis

Data are expressed as means ± SE. Data were analyzed statistically using the paired Student *t* test. Probability values of less than 0.05 were considered statistically significant.

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Results

Expression of hOCT3 mRNA in normal and cancerous colorectal tissues

The expression of hOCT3 mRNA in colon (n=6) or rectum (n=10) tissue derived from Japanese patients was measured by real-time PCR. Figure 1 shows the difference in the expression between normal and cancerous colorectum. In cancerous colon tissue, the level of hOCT3 mRNA was significantly higher than that in normal tissue, and the mean increase in individuals was 9.7-fold (Fig. 1A). The median value of hOCT3 in normal and cancerous colon tissue was 0.44 (range; 0.24-1.59) and 5.59 (range; 0.20-8.62) zmol/μg of total RNA, respectively (P=0.0247, by the paired Student *t* test). hOCT3 mRNA expression in rectum tended to increase in cancerous tissue, but the difference was not significant (Fig. 1B). The median value of hOCT3 in normal and cancerous rectum tissue was 0.87 (range; 0.39-5.17) and 1.26 (range; 0.41-17.1) zmol/μg of total RNA, respectively (P=0.363, by the paired Student *t* test).

mRNA expression of organic cation transporters in colorectal cancer-derived cell lines

We examined the expression of hOCT1, hOCT2, hOCT3, hMATE1 and hMATE2-K mRNA in colorectal cancer-derived cell lines, T84, SW480, HCT116, HT29, SW837 and Lovo, by real-time PCR. The hOCT3 transcript was found in all these cells except HCT116, and was strongly detected in T84 and SW837 (Fig. 2). The hMATE1 mRNA was only expressed in SW480. However, the mRNA expression of hOCT1, hOCT2, hMATE1 and hMATE2-K in these cells was almost negligible.

[^{3}H]MPP uptake by colorectal cancer-derived cell lines

To check the functional activity of hOCT3 in cultured cells, we measured the cellular uptake of its typical substrate, [^{3}H]MPP. The accumulation of [^{3}H]MPP was greater in T84,

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SW480 expressing hOCT3, and HEK293 expressing hOCT3 than in SW480, SW480 transfected with vector cDNA, and HEK293 transfected with vector cDNA (Fig. 3A). In addition, we examined [^{3}H]MPP uptake in other colorectal cancer-derived cell lines, HCT116, HT29, SW837 and Lovo. SW837 showed the highest level of activity to transport [^{3}H]MPP among these six cell lines (Fig. 3B). The transport activity of the cells was confirmed, and then, these cells and expression systems were used in subsequent experiments on the cytotoxicity and the cellular transport of platinum agents.

hOCT3 expression and oxaliplatin-induced cytotoxicity

We examined the effect of hOCT3 expression in a colon cancer-derived cell line, SW480. When SW480 cells transfected with 800 ng of empty vector or hOCT3 were treated with 500 μM oxaliplatin for 6 h and subsequently cultured in normal medium for 24 h, the release of LDH into the culture medium was increased by the expression of hOCT3 (Fig. 4A). In addition, we measured the amount of LDH released by the treatment with 500 μM oxaliplatin in other colorectal cancer-derived cell lines. The amount of released was the greatest in SW837, and was also large in T84 and Lovo (Fig. 4B). SW480, HCT116 and HT29 showed little release of LDH on the treatment with oxaliplatin.

Moreover, the caspase 3/7 activity induced by the treatment with 50 μM oxaliplatin was examined in these cell lines. The most potent activation of caspase 3/7 was in SW837, but T84 and Lovo also showed strong caspase 3/7 activity (Fig. 4C). The results of caspase activity were consistent with those of LDH release.

Transport of oxaliplatin

We examined the accumulation of oxaliplatin with the increase of hOCT3 cDNA on transfection of SW480 cells (Fig. 5A), because almost no hOCT3 mRNA was found in

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SW480 (Fig. 2). When SW480 cells transfected with 50-800 ng of hOCT3 cDNA per well were treated with 1000 μM oxaliplatin for 1 h, the level of platinum accumulated in the cells was increased dependent on the amount of hOCT3 cDNA transfected (Fig. 5A). Based on these results, we determined the platinum accumulation in T84, SW480 and SW480 transfected with 800 ng of hOCT3 cDNA. When treated with 100, 500 or 1000 μM oxaliplatin for 1 h, T84 and SW480 expressing hOCT3 extensively transported oxaliplatin in a concentration-dependent manner compared with SW480 or SW480 transfected with empty vector (Fig. 5B). Moreover, we examined the amount of platinum accumulated after the treatment with oxaliplatin in other colorectal cancer-derived cell lines, HCT116, HT29, SW837 and Lovo. Platinum was most abundant in SW837 at all three concentrations when the cells were incubated with the culture medium containing oxaliplatin for 2 min (Table 1). The same tendency was observed when they were treated for 1 h (Table 2). In HT29 and Lovo, the amount of platinum accumulated was approximately half of that in SW837, and the level in SW480 and HCT116 was low compared with that in other cultured cells.

Relation among hOCT3 mRNA expression, LDH release, and platinum accumulation

When cultured cells were treated with 500 μM oxaliplatin, the release of LDH was increased by the hOCT3 mRNA expression (Fig. 6A). The accumulation of platinum in the cells after the incubation with 500 μM oxaliplatin was also dependent on the hOCT3 mRNA expression (Fig. 6B). Combining the data from Fig. 6a and Fig. 6b, the release of LDH was also comparable with the accumulation of platinum (Fig. 6C). On the other hand, when cells were treated with 500 μM cisplatin, the accumulation of platinum was independent of the hOCT3 mRNA expression (Fig. 6D).

Cancer profiling array

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We examined the differences in hOCT3 expression between normal and cancerous tissues derived from Caucasians using dot blotting, and the density of each dot was quantified using ImageJ 1.38x (Fig. 7). In the colon, the level of hOCT3 was significantly higher in the cancerous tissues (Fig. 7A). This result was consistent with that in Fig. 1a. A significant increase of hOCT3 expression was also observed in the rectum and stomach (Figs. 7B and 7C). Inversely, a significant decrease of hOCT3 expression in cancerous tissue was detected in the uterus, breast, ovary, and lung (Figs. 7D-7G). In the kidney, there was no significant difference of hOCT3 mRNA expression between normal and cancerous tissue (Fig. 7H).

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Discussion

Oxaliplatin has a much more potent anti-colorectal cancer effect than cisplatin (Grem et al., 1993; de Gramont et al., 2000). However, the molecular mechanism(s) which cause the difference in the effect has not been made clear. We previously elucidated that oxaliplatin, but not cisplatin, was transported by OCT3 (Yonezawa et al., 2006; Yokoo et al., 2007). Therefore, we hypothesized that the level of hOCT3 in cancerous tissues contributed to the superior anticancer effect of oxaliplatin. In the present study, the level of hOCT3 mRNA was significantly higher in cancerous colon than in normal colon tissues derived from Japanese patients (Fig. 1A). In addition, this tendency was reproduced in Caucasians using a cancer profiling array (Figs. 7A and 7B). These findings indicated that the level of hOCT3 mRNA was heightened by colorectal cancerous transformation independent of ethnicity.

In human colorectal cancer-derived cell lines, hOCT3 mRNA expression was correlated with the release of LDH and accumulation of platinum induced by the treatment with oxaliplatin (Figs. 6A and 6B). These results suggested that hOCT3 expression is a candidate marker for the efficacy of oxaliplatin treatment. Moreover, the release of LDH and accumulation of platinum caused by the incubation with cisplatin was independent of the hOCT3 mRNA level (Fig. 6D). This result was consistent with the report that cisplatin was not transported by hOCT3 (Yonezawa et al., 2006). Therefore, hOCT3 expression is suggested to be closely associated with the anticancer activity of oxaliplatin, but not of cisplatin.

Cisplatin plays an essential role in chemotherapy against solid tumors of the prostate, bladder, lung, testis, liver and brain (Ho et al., 2003). However, the effect of cisplatin on colorectal cancer is weak. Loehrer et al. (Loehrer et al., 1988) and Grem et al. (Grem et al., 1993) reported that the rate of response of colorectal cancer to cisplatin-based chemotherapy was 22% and 19%, respectively. On the other hand, for oxaliplatin-based chemotherapy, de

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Gramont et al. (de Gramont et al., 2000) reported that the response rate was 50%. The differences of molecular mechanisms whereby cisplatin has a weak effect but oxaliplatin has a strong effect on colorectal cancer have been unclear. The anticancer activity and resistance to platinum agents have been considered to be related with the DNA repair pathway, nucleotide-excision repair, base-excision repair mismatch repair, and double-strand-break repair, or the substrate specificity of copper transporters, CTR1, ATP7A, and ATP7B (Kelland, 2007). However, recently, we and others reported the contribution of organic cation transporters in the cellular transport of platinum agents (Ciarimboli et al., 2005; Yonezawa et al., 2005; Yonezawa et al., 2006; Zhang et al., 2006; Yokoo et al., 2007; Kitada et al., in press). Zhang et al. (Zhang et al., 2006) reported that the effect of oxaliplatin against colon cancer was related to the expression of hOCT1 and hOCT2. Kitada et al. (Kitada et al., in press) reported that the level of ATP7A, and hOCT1 mRNA affects the sensitivity to oxaliplatin. However, we reported that oxaliplatin was transported by both human and rat OCT2 and OCT3, but not by OCT1 (Yonezawa et al., 2006; Yokoo et al., 2007). In the present study, only hOCT3 mRNA was found in the six cell lines derived from colorectal cancers, and the cytotoxicity of oxaliplatin was associated with the expression level. Based on these findings and the present results, at least in colorectal cancer, OCT3 is thought to be important for sensitivity to oxaliplatin.

In the six colorectal cancer-derived cell lines, hOCT3 mRNA levels were markedly higher than hOCT1, hOCT2, hMATE1, or hMATE2-K mRNA levels (Fig. 2). Previously, we reported that oxaliplatin was also transported by hOCT2 (Yonezawa et al., 2006). However, in these cell lines, the expression of hOCT2 mRNA was little detected by real-time PCR (Fig. 2). Therefore, the contribution of hOCT2 to the anticancer effect of oxaliplatin was suggested to be small. The transport activity of hOCT3 in these cells was confirmed by using [^{3}H]MPP, a typical substrate of OCT3 (Fig. 3). Okuda et al. (Okuda et al., 2000)

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reported that the cytotoxicity of cisplatin differed at low and high doses, that is, 30 μM and 1000 μM of cisplatin induced apoptosis and necrosis, respectively. In the present study, we used two indexes of cytotoxicity, LDH release and caspase 3/7 activity, as indicators of necrosis and apoptosis, respectively. Both LDH release and caspase activity showed a similar tendency, that is, values were high in cell lines expressing high levels of hOCT3 mRNA (Figs. 2, 4B and 4C). From these results, the hOCT3-mediated cellular accumulation of oxaliplatin might be a trigger for the subsequent cytotoxic effects.

Although the cytotoxicity of oxaliplatin in T84 cells was lower than expected given the expression level of hOCT3 (Fig. 6A), the LDH release in these cells correlated quite well with the platinum accumulation (Fig. 6C). These results suggest that some mechanisms including unknown oxaliplatin efflux transporter, which reduces the intracellular platinum concentration in T84 cells compared to SW837 cells.

We had reported that the nephrotoxicity caused by treatment with platinum agents was closely associated with their renal accumulation, which is determined by the substrate specificity of the OCT and MATE families (Yonezawa et al., 2005; Yonezawa et al., 2006; Yokoo et al., 2007). There had also been a report that the uptake of imatinib, a tyrosine kinase inhibitor effective in the treatment of chronic myeloid leukemia (CML), was mediated by hOCT1 (Thomas et al., 2004). Recently, two groups showed that hOCT1 was a determinant of outcome in imatinib-treated CML (White et al., 2007; Wang et al., 2008). Patients with a high level of hOCT1 had a greater probability of achieving a cytogenetic response and a superior progression-free and overall survival (Wang et al., 2008). These reports showed the participation of hOCT1 in the clinical effects of imatinib. Therefore, the results of this study, that the cytotoxicity of oxaliplatin depended on hOCT3 expression, may be expanded to include the effectiveness in clinical cases.

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OCT3 is widely distributed in many tissues (Kekuda et al., 1998), but its function has been mainly examined in the brain (Wu et al., 1998; Gasser et al., 2006). The results of this study suggested a new role for OCT3, as a determinant of the sensitivity of treatment with oxaliplatin against colorectal cancer. At present, oxaliplatin is used for colorectal cancer as a key drug of FOLFOX regimens (de Gramont et al., 2000). Other combinations including oxaliplatin for colorectal cancer or other cancers have been used in clinical trials (Goldberg et al., 2004; Zhu et al., 2006). The level of hOCT3 in cancerous tissue was significantly higher in colon, rectum, and stomach (Figs. 7A-7C). Conversely, the level was significantly lower in uterus, breast, ovary, and lung (Figs. 7D-7G). These changes of hOCT3 expression might contribute to the sensitivity and selectivity of oxaliplatin-based chemotherapy. Recently, there were several reports that oxaliplatin was effective against gastric cancer in phase II trials (Lordick et al., 2005; Park et al., 2006; Kim et al., 2008). Considering the present results, there is a possibility that the increase of hOCT3 expression in cancerous tissue affects the results of clinical trials. Therefore, taking a positive attitude to using oxaliplatin-based chemotherapy for other cancers, which express high levels of hOCT3 compared to normal tissue, may lead to good clinical results.

In the present study, we clearly found that the selective induction of hOCT3 mRNA expression in colon cancer and colorectal cancer-derived cell lines. The cytotoxicity and accumulation of platinum caused by the treatment with oxaliplatin, but not cisplatin, depended on the expression of hOCT3 mRNA. In conclusion, the uptake of oxaliplatin into the cancer cells via hOCT3 was suggested to be an important mechanism for its cytotoxicity, and the expression of hOCT3 in cancers may became a marker for including oxaliplatin in cancer chemotherapy.

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Footnotes

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Legends to Figures

Figure 1. hOCT3 mRNA expression in normal or cancerous tissues derived from Japanese patients. The expression of hOCT3 mRNA in colon (A) or rectum (B) was detected by real-time PCR. The number of patients with colon and rectum cancer was six and ten, respectively. The horizontal bars represent the median of hOCT3 mRNA expression.

Figure 2. mRNA levels of organic cation transporters in colorectal cancer-derived cell lines. Total RNA from the cell lines was reverse-transcribed, and the OCT1, hOCT2, hOCT3, hMATE1, or hMATE2-K expression level was measured by using real-time PCR. Each column represents the mean ± SE for three wells. The plot of real-time PCR results for hOCT3 and GAPDH is in supplemental data.

Figure 3. Uptake of [^{3}H]MPP by colorectal cancer-derived cell lines or HEK293. After preincubation, native T84 or SW480, and SW480 or HEK293 transiently transfected with empty vector (vector) or hOCT3 cDNA (A), or T84, SW480, HCT116, HT29, SW837 or Lovo (B) was incubated with 13.7 nM [^{3}H]MPP for 2 min at 37°C. Each column represents the mean ± SE for three wells.

Figure 4. Role of hOCT3 expression in the oxaliplatin-induced cytotoxicity. SW480 transiently expressing hOCT3 or empty vector (A), or T84, SW480, HCT116, HT29, SW837 or Lovo (B) was treated with 500 μM oxaliplatin in the culture medium for 6 h. Then, the cells were incubated in the normal culture medium for 24 h. The amount of LDH released into the medium was measured. (C) T84, SW480, HCT116, HT29, SW837 or Lovo was treated with 50 μM oxaliplatin in the culture medium for 8 h, and then, caspase 3/7 activity

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was measured. Each column represents the mean ± SE for three wells.

Figure 5. Uptake of oxaliplatin by colorectal cancer-derived cell lines. (A) SW480 was transfected with an amount of hOCT3 cDNA and vector plasmid added to 800 ng using 2 µl of Lipofectamine 2000. The cells were exposed to 1000 µM oxaliplatin in the culture medium for 1 h. (B) T84, SW480 or SW480 transiently expressing empty vector or hOCT3 was treated with culture medium containing 100, 500, or 1000 µM oxaliplatin for 1 h. After being washed, these cells were solubilized in 0.5 N NaOH, and the amount of platinum was determined by ICP-MS. Each point represents the mean ± SE of four wells.

Figure 6. Relation among hOCT3 mRNA expression, LDH release, and platinum accumulation. The data on mRNA expression are from Fig. 2, and the data on the release of LDH and accumulation of platinum are from Fig. 4b and Table 2, respectively. (A) hOCT3 mRNA expression versus LDH release on treatment with 500 µM oxaliplatin, (B) hOCT3 mRNA expression versus platinum accumulation on treatment with 500 µM oxaliplatin, (C) platinum accumulation versus LDH release on treatment with 500 µM oxaliplatin, and (D) hOCT3 mRNA expression versus platinum accumulation on treatment with 500 µM cisplatin.

Figure 7. The differences in hOCT3 expression between normal and cancerous tissues. The differences of hOCT3 expression between normal and cancerous tissue were examined by dot blotting. The density of each dot was quantified using ImageJ 1.38x. Figures represent the colon (A, n=39), rectum (B, n=18), stomach (C, n=23), uterus (D, n=42), breast (E, n=35), ovary (F, n=14), lung (G, n=20) and kidney (H, n=11). The bars represent median values.

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TABLE I – Platinum accumulation in colorectal cancer-derived cell lines (2 min)

Cell lines	Oxaliplatin 100 μM	Oxaliplatin 500 μM	Oxaliplatin 1000 μM
		Platinum accumulation (ng/mg protein/2 min)	
T84	0.82 ± 0.03	5.12 ± 0.06	9.77 ± 0.46
SW480	0.48 ± 0.01	2.27 ± 0.02	4.19 ± 0.04
HCT116	0.46 ± 0.03	2.72 ± 0.08	5.23 ± 0.26
HT29	0.64 ± 0.01	3.55 ± 0.05	7.85 ± 0.19
SW837	1.07 ± 0.02	5.77 ± 0.10	14.3 ± 1.13
Lovo	0.61 ± 0.01	3.59 ± 0.07	7.27 ± 0.10

Colorectal cancer-derived cell lines were treated with medium containing 100, 500, or 1000 μM oxaliplatin for 2 min. After being washed, these cells were solubilized in 0.5 N NaOH, and the amount of platinum was determined by ICP-MS. Each value represents the mean ± SE for four wells.

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TABLE II – Platinum accumulation in colorectal cancer-derived cell lines (1 h)

Cell lines	Oxaliplatin 100 μM	Oxaliplatin 500 μM	Oxaliplatin 1000 μM
		Platinum accumulation (ng/mg protein/h)	
T84	14.0 ± 0.3	70.5 ± 1.2	160.2 ± 3.9
SW480	4.9 ± 0.1	25.0 ± 0.5	64.0 ± 1.4
HCT116	6.0 ± 0.2	32.3 ± 0.6	79.2 ± 2.8
HT29	8.7 ± 0.1	47.8 ± 0.1	123 ± 2.6
SW837	16.7 ± 0.4	91.6 ± 2.3	222 ± 3.8
Lovo	9.6 ± 0.0	52.4 ± 1.2	126 ± 2.1

Colorectal cancer-derived cell lines were treated with medium containing 100, 500, or 1000 μM oxaliplatin for 1h. After being washed, these cells were solubilized in 0.5 N NaOH, and the amount of platinum was determined by ICP-MS. Each value represents the mean ± SE for four wells.

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Figure 1

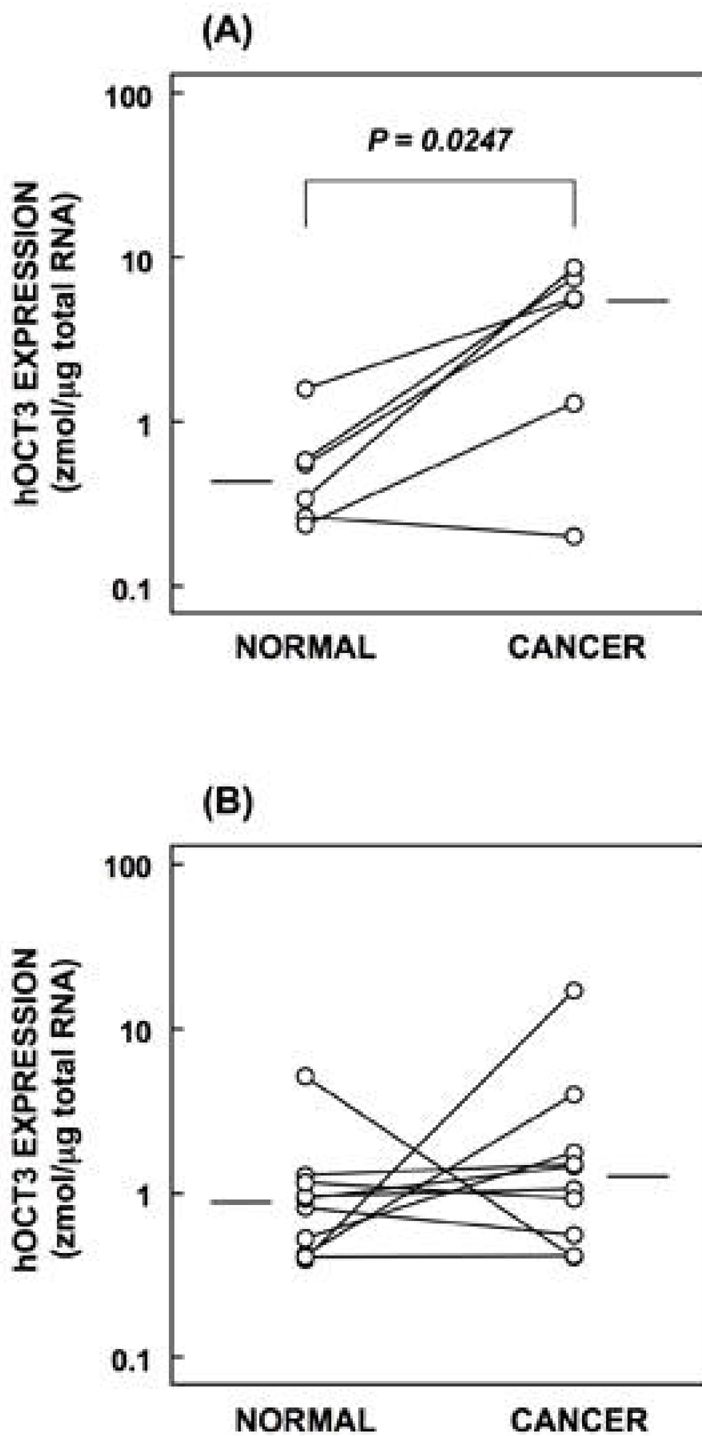

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Figure 2

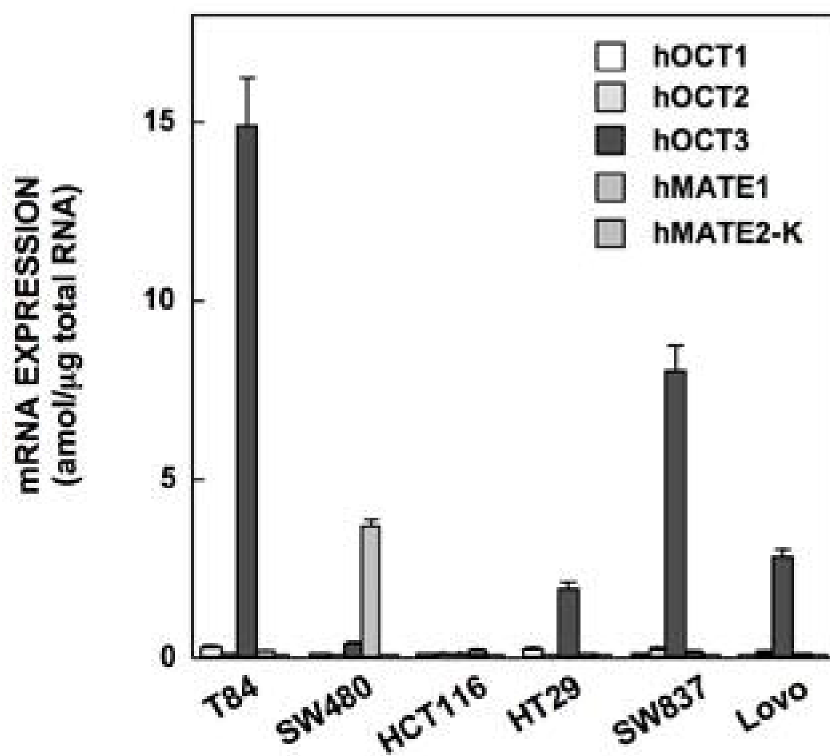

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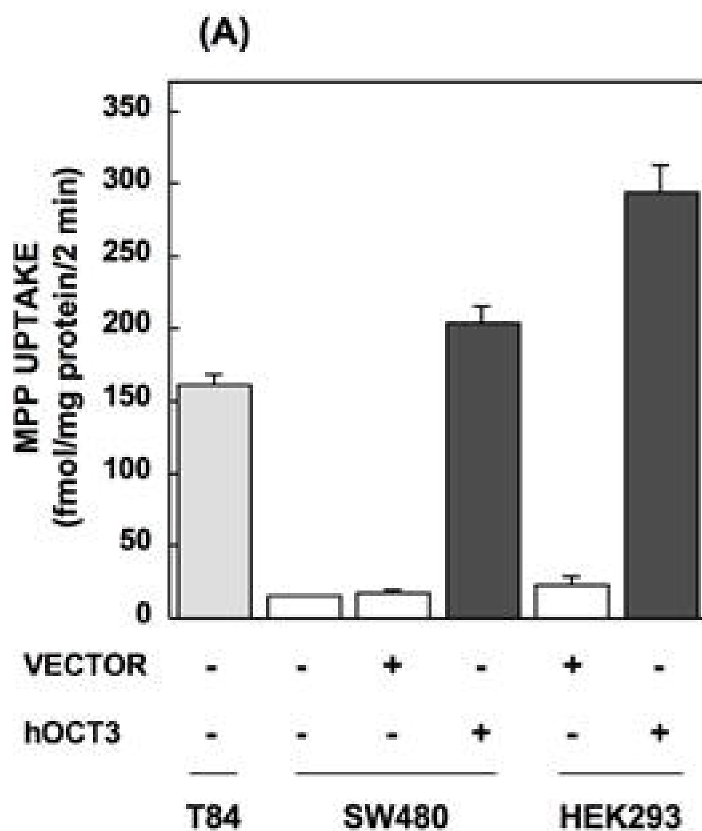
Figure 3



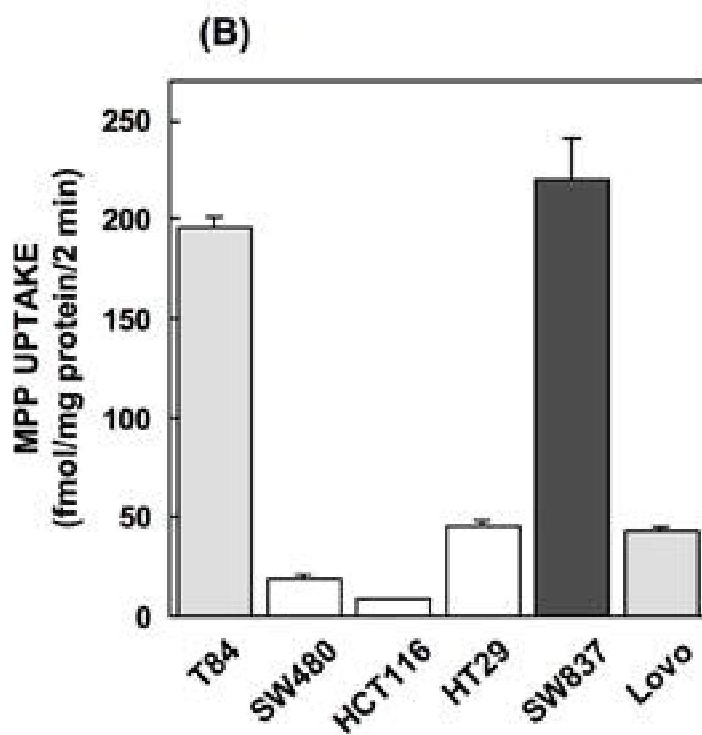


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Figure 4

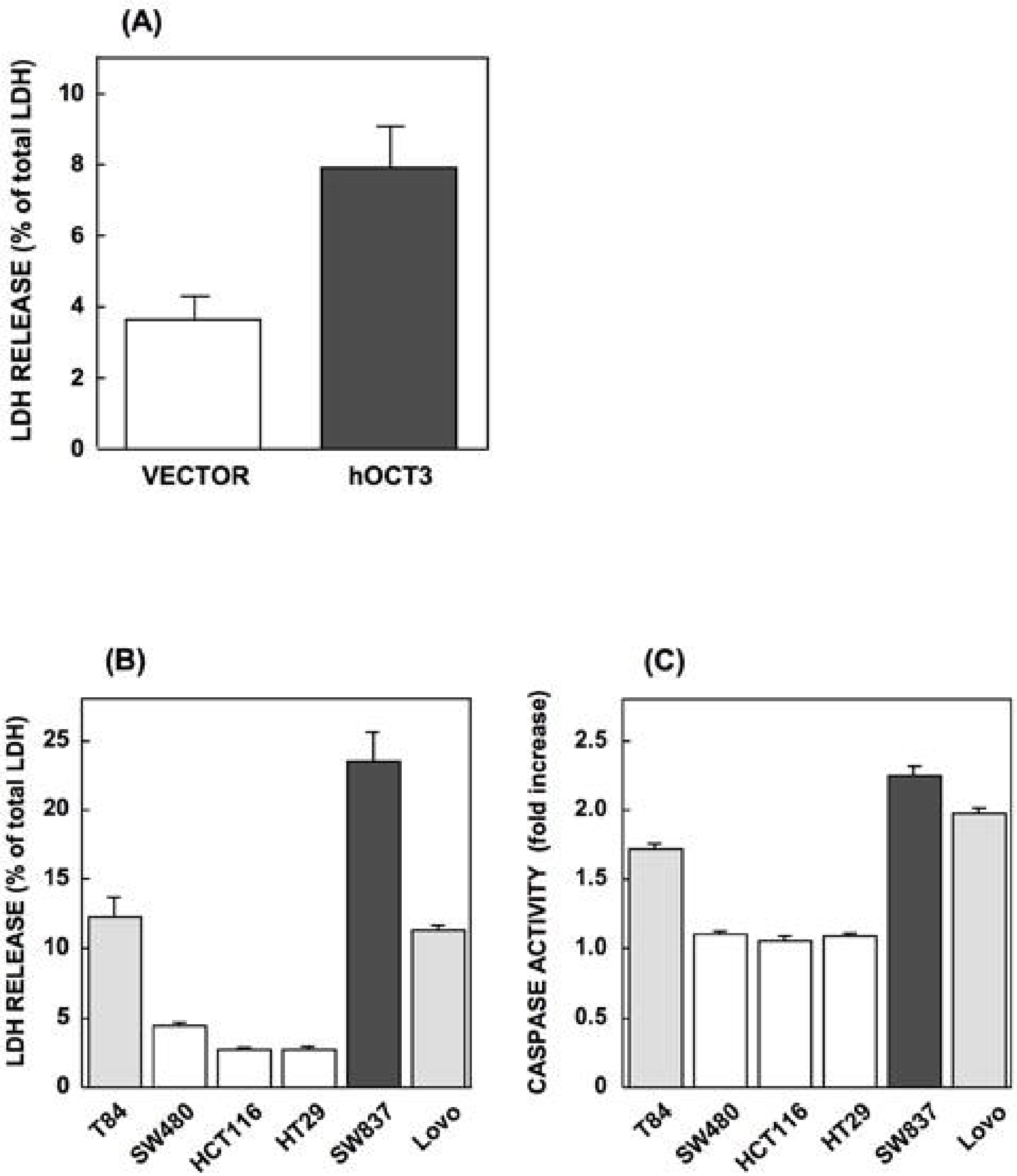

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Figure 5

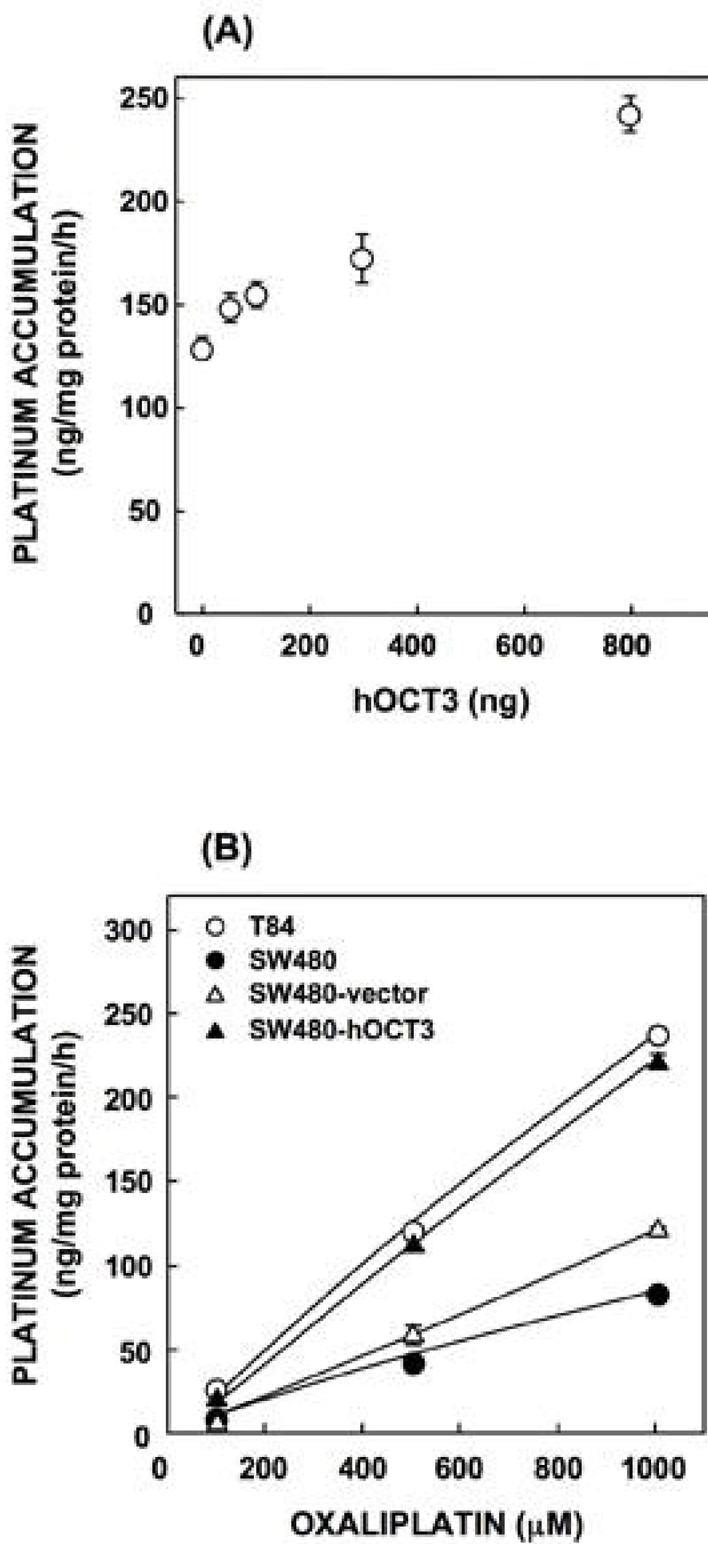

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Figure 6

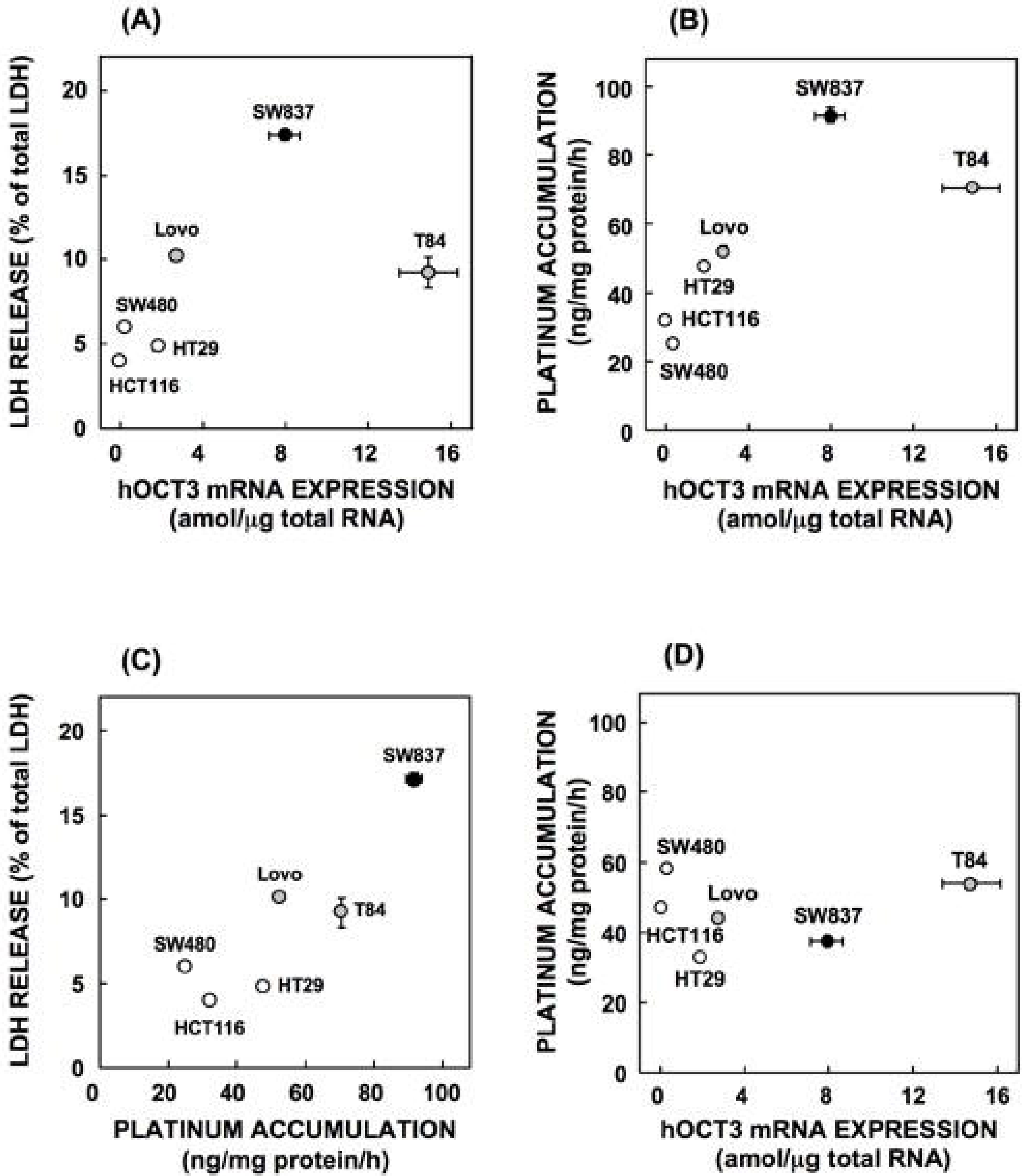

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Figure 7