Role and mechanism of organic cation transporter 3 in oxaliplatin treatment of colon cancer \textit{in vitro} and \textit{in vivo}

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\textbf{Abstract.} Oxaliplatin (OXA) is routinely used as the first-line treatment for colorectal cancer (CRC). The addition of OXA to chemotherapy has significantly improved the prognosis of patients with CRC; however, some cases are resistant to OXA. The present study explored the influence of organic cation transporter 3 (OCT3) expression on the effects of OXA on CRC cell viability, and investigated the direct effects of OCT3 on viability, invasion and migration of CRC cells using MTT assay, wound healing assay, reverse transcription-quantitative polymerase chain reaction, inductively coupled plasma mass spectrometry and lentiviral interference. The results demonstrated that OXA cellular concentration and OXA-induced cytotoxicity were significantly increased in response to high expression of OCT3, whereas OCT3 knockdown directly increased the invasion and migration of colon cancer cells. Furthermore, upregulation of OCT3 expression in colon cancer xenografts via treatment with the DNA methyltransferase inhibitor decitabine increased cellular OXA concentration and improved the curative effect of OXA. These results collectively indicated that OCT3 may enhance the effects of OXA in CRC cells and may directly inhibit their invasion and migration. Therefore, OCT3 may be a therapeutic target in patients with CRC.

\textbf{Introduction.} Oxaliplatin (OXA) is a widely used third-generation platinum anticancer drug that is becoming the standard-of-care in the management of colorectal cancer (CRC). Conversely, other platinum-based drugs lack anticancer efficacy in CRC (1). However, many patients do not benefit from OXA treatment (2). Buss \textit{et al} (3) indicated that a reduction in the influx rate of OXA has been observed in resistant CRC cell lines; however, no difference in efflux rate exists between sensitive and resistant CRC cell lines. In addition, Oguri \textit{et al} (4) demonstrated that the intracellular accumulation of OXA in PC-14 cells resistant to OXA is reduced compared with in parental cells; however, no difference has been reported in the expression of efflux transporters SLC47A1 and SLC47A2. Organic cation transporter 3 (OCT3), which belongs to the solute carrier family and is encoded by solute carrier family 22 member 3 (\textit{SLC22A3}), is critical for drug transportation and cellular detoxification (5,6). The expression of OCT3 in CRC cell lines is higher than that of other organic cation transporters, and the higher the expression of OCT3 in CRC cell lines, the higher the concentration of OXA in the cells (7), suggesting that OCT3 may have a role in the uptake of OXA, although the results are contradictory (8). Whether the expression and regulation of OCT3 influences the effect of OXA on CRC and the possible mechanisms remain to be investigated. A recent study suggested that \textit{SLC22A3} may be a tumor suppressor gene (9-12). Fu \textit{et al} demonstrated that \textit{SLC22A3} suppresses esophageal squamous cell carcinoma metastasis by inhibiting epithelial-mesenchymal transition (EMT) (12). Guo \textit{et al} reported that OXA-resistant HCT116 CRC cells exhibit an EMT phenotype characterized by upregulated expression of matrix metalloproteinase (MMP)2 and MMP9, and downregulated E-cadherin expression (13). Further investigation is required as to whether OCT3 can increase the effects of OXA on CRC by inhibiting the EMT of CRC cells. CRC is the third most common cancer worldwide (14). At diagnosis, ~20% of patients with CRC have distant metastases, with 30-40% having developed vascular and lymph node metastasis (15). Therefore, the discovery of targets that can affect the malignant behavior of CRC is of great significance in developing novel preventative and treatment strategies.
Aberrant DNA methylation is a potential mechanism underlying the development of CRC and reflects the chemosensitivity of patients receiving postoperative adjuvant chemotherapy (16-19). DNA methylation is an epigenetic process that can lead to silencing of gene expression and can be reversed by DNA-demethylating agents. Analysis of SLC22A3 using MethPrimer (20) revealed that CpG islands exist in its promoter region, suggesting that regulation of this gene by methylation may have a role in its expression. The DNA methyltransferase inhibitor decitabine (DAC) is a Food and Drug Administration-approved drug used clinically to treat acute myelodysplastic leukemia, and has been reported to inhibit cell invasiveness and proliferation of CRC lines (21).

The present study aimed to explore the effect and possible mechanisms of OCT3 in OXA treatment of CRC in vitro and in vivo. Specifically, OCT3 expression was induced in HCT116 cells with low OCT3 expression using DAC, and SLC22A3 expression was knocked down in HT29 cells with high OCT3 expression via stable lentiviral interference, in order to investigate the effects of OCT3 expression on OXA transport and CRC cell viability. The direct effect of OCT3 on malignant biological behaviors, such as migration and invasion of CRC cells, was investigated using a wound healing assay and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). In addition, the curative effect of DAC combined with OXA in a nude mice xenograft model of CRC was explored and the association between OCT3 expression and OXA concentration was investigated. The concentration of OXA was detected by inductively coupled plasma mass spectrometry (ICP-MS).

Materials and methods

**Materials.** OXA was purchased from Dalian Meilun Biotechnology Co., Ltd. DAC (5-Aza-2’-deoxycytidine) was purchased from Selleck Chemicals and MTT was purchased from Sigma-Aldrich (Merck KGaA). Solutions of OXA (7 mmol/l) and DAC (40 mmol/l) were freshly prepared in DMEM cell culture media (Gibco; Thermo Fisher Scientific, Inc.).

**Cell culture.** CRC cell lines HCT116, HT29, SW620 and 293T were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. HCT116, HT29 and 293T cells were cultured in high glucose DMEM (Gibco; Thermo Fisher Scientific, Inc.), and SW620 cells were cultured in Leibovitz’s L-15 (Gibco; Thermo Fisher Scientific, Inc.) according to the manufacturer’s protocol at a ratio of 1:1 to produce the lentivirus. 293T cells were incubated at 37°C and 5% CO₂ for 48 h. The supernatant was collected 48 h post-transfection, centrifuged at 4°C and 1,500 x g for 10 min, and filtered through a 0.45-μm microporous membrane to remove cell debris, and temporarily stored at 4°C. A total of 4x10⁶ human CRC cell lines (HCT116 and HT29) were inoculated in a 12-well plate and infected with the viral suspension (100 μl crude viral liquid per well) alongside 6 μg/ml polybrene [Yeasen Biotechnology (Shanghai) Co., Ltd.]; the optimal lentivirus volume was determined through preliminary experiments. A total of 72 h post-infection, the positive stably transduced cell lines were screened using puromycin (Thermo Fisher Scientific, Inc.) at 1 μg/ml for HCT116 cells and 0.5 μg/ml for HT29 cells. RT-qPCR was employed to detect interference efficiency.

**Cell treatment.** For RT-qPCR analysis, a total of 2x10⁵ cells were seeded into 6-well plates, 6 h after which, DAC was added at different density (0.6, 1.25, 2.5, 5 and 50 μM for HCT116 cells; 0.63, 1.25, 2.5, 5 and 50 μM for SW620 cells; 2.5, 20, 50 and 100 μM for HT29 cells) for 72 h. In addition, cells of different concentrations were inoculated into 6-well plates, 2.5 μM DAC was added to HCT116 cells for 24 h (6x10⁵ cells per well), 48 h (4x10⁵ cells per well) and 72 h (2x10⁵ cells per well), and to SW620 and HT29 cells for 48 h (4x10⁵ cells per well), 72 h (2x10⁵ cells per well) and 96 h (1x10⁵ cells per well). Cells were incubated at 37°C and 5% CO₂.

**RT-qPCR analysis.** Total RNA was isolated from cultured cells (80% confluent) using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. First-strand cDNA was synthesized using a reverse transcriptase kit (cat. no. TAKARA047A; Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. Subsequently, qPCR was conducted according to the manufacturer's protocols under the following conditions: Denaturation for 5 min at 95°C, followed by 40 cycles of 15 sec at 95°C, 20 sec at 60°C and 30 sec at 72 °C, and a final extension step of 5 min at 72°C on a Linegene 9620 real-time PCR system (Hangzhou Biocer Technology Co., Ltd.) using SYBR-Green master mix [Yeasen Biotechnology Company, Ltd.]. The shRNA negative control sequence, which had no significant homology to human gene sequences, was 5’-CAACAGATGAAGAGCACAAG-3’. The shRNA sequence targeting SLC22A3 (sh-OCT3) was 5’-GAGGAA ATGCACCTATTTCT-3’ for HCT116 cells and 5’-GAATTG TACCCCAAAACATTA-3’ for HT29 cells. These shRNA fragments were cloned into pLV-shRNA-GP vector-Puro (Shanghai Yile Biotechnology Company, Ltd.) to construct lentiviral vectors. A total of 3x10⁵ 293T cells were inoculated in a 6 cm culture dish 1 day prior to transfection. The cells were cultured overnight in a 37°C and 5% CO₂ to ensure 80-90% cell confluence at the time of transfection. Subsequently, the lentiviral vector (0.6 μg/ml) and auxiliary plasmids (0.6 μg/ml; Gag-Pol:Rev:SVG ratio of 5:2:3; Shanghai Yile Biotechnology Company, Ltd.) were transferred into 293T cells using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer’s protocol at a ratio of 1:1 to produce the lentivirus. 293T cells were incubated at 37°C and 5% CO₂ for 48 h. The supernatant was collected 48 h post-transfection, centrifuged at 4°C and 1,500 x g for 10 min, and filtered through a 0.45-μm microporous membrane to remove cell debris, and temporarily stored at 4°C. A total of 4x10⁶ human CRC cell lines (HCT116 and HT29) were inoculated in a 12-well plate and infected with the viral suspension (100 μl crude viral liquid per well) alongside 6 μg/ml polybrene [Yeasen Biotechnology (Shanghai) Co., Ltd.]; the optimal lentivirus volume was determined through preliminary experiments. A total of 72 h post-infection, the positive stably transduced cell lines were screened using puromycin (Thermo Fisher Scientific, Inc.) at 1 μg/ml for HCT116 cells and 0.5 μg/ml for HT29 cells. RT-qPCR was employed to detect interference efficiency.
Gene-specific primers were synthesized by the Beijing Genomics Institute and are listed in Table I. All samples were normalized against ACTB expression. Relative gene expression was analyzed using the $2^{\Delta\Delta Cq}$ method (22).

Western blotting. Cells were lysed in ice-cold RIPA lysis buffer (Beyotime Institute of Biotechnology) containing PMSF, and protein concentrations were detected using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). A total of 40 µg protein was separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (EMD Millipore), which were blocked by soaking in 5% non-fat milk for 1.5 h at 37˚C. Subsequently, the membranes were incubated with polyclonal rabbit anti-human OCT3 antibody (1:500; cat. no. OM291394; OminimAbs) or polyclonal rabbit anti-human GAPDH antibody (1:1,000; cat. no. WL01114; Wanleibio Co., Ltd.) overnight at 4˚C. After washing with 1X TBS-0.1% Tween three times (10 min/wash), the membranes were incubated with goat anti-rabbit immunoglobulin G/horse radish peroxidase (1:10,000; cat. no. bs-0295G-HRP; BIOSS) for 1 h at 37˚C. Finally, the bands were visualized using an enhanced chemiluminescence kit (EMD Millipore). Signals were semi-quantified by ImageJ software (version 1.8.0; National Institutes of Health) and normalized to GAPDH.

Cell viability assay. The cytotoxicity of compounds was examined using the MTT assay (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were seeded into 96-well plates at ~5,000 cells per well, incubated overnight, and treated with 2.5 µM DAC for the following durations: HCT116 for 24, 48 and 72 h; SW620 cells for 48 and 72 h; and HT29 cells for 72 h. OXA was added at different concentrations (0.625, 1.25, 2.5, 10, 20, 50, 200, 800, 3,200 and 6,400 µM for HCT116 cells; 1.25, 2.5, 10, 50, 75, 100, 200, 600, 1,200 and 2,400 µM for SW620 cells; 0.625, 2.5, 5, 10, 25, 75, 150, 300, 350, 400 and 650 µM for HT29 cells) for a further 24 h, and 10 µl MTT reagent was then added to each well and incubated at 37˚C for 4 h. After gently removing the culture medium from the 96-well plates, 100 µl dimethyl sulfoxide was added to the 96-well plates, and the crystals were dissolved in a shaker at low speed at 37˚C for 10 min. An automatic microplate spectrophotometer (Multiskan MK3; Thermo Fisher Scientific, Inc.) was used to measure absorbance at 490 nm. All experiments were repeated at least three times.

Cell migration assay. The bottoms of 9.5 cm² dishes were marked with a pen and ruler prior to cell seeding. Subsequently, control, negative control and sh-OCT3 HT29 cells (1.6x10⁵ cells) were seeded into 9.5 cm² dishes in 1% serum-containing medium, and allowed to reach 90-100% confluence overnight, forming a monolayer. Subsequently, the cells were scratched with a 10-µl pipette tip perpendicularly to the surface to cross the marker lines, forming fixed detection points. Cells were washed with PBS twice followed by the addition of fresh media with 1% FBS. Images were captured at 0, 24, 36 and 48 h using an inverted light microscope.

Methylation-specific PCR (MSP) assays. The promoter region of SLC22A3 was searched for in Genecopoeia (www.genecopoeia.com). MSP primers, as detailed in Table II, were designed using MethPrimer (The Li Lab, www.urogene.org/methprimer) for the promoter region of SLC22A3. Genomic DNA extraction and purification was performed using the Genomic DNA Small Purification kit (Wanleibio Co., Ltd.).

| Table I. Primers for reverse transcription-quantitative polymerase chain reaction analysis. |
|---------------------------------|-----------------|-----------------|-----------------|
| **Oligonucleotide name** | **Source** | **Sequence (5'-3')** | **Amplified fragment length (bp)** |
| SLCC2A3-F | Human | CCCTGGGAATTGCCTACTTCA | 102 |
| SLCC2A3-R | Human | GACTCAAGGGACCCCAAGTA | 211 |
| β-actin-F | Human | CATCGAGCCAGGCATCGTCA | |
| β-actin-R | Human | TAGGACACGCTGGAGCATCAAC | |
| MMP2-F | Human | GATACCCTTTGTACCGTAAAGGA | 112 |
| MMP2-R | Human | CCTTCTCCCAAGGTCCATAGC | |

F, forward; R, reverse; MMP2, matrix metalloproteinase 2; SLC22A3, solute carrier family 22 member 3.

| Table II. Primers for methylation-specific polymerase chain reaction analysis. |
|---------------------------------|-----------------|-----------------|-----------------|
| **Oligonucleotide name** | **Source** | **Sequence (5'-3')** |
| SLCC2A3 M-F | Human | TATATGGGCGTAGGAGGTTTC |
| SLCC2A3 M-R | Human | AAACCCGATCTCTCAACGAC |
| SLCC2A3 U-F | Human | TTTTATAGGGTGTAGGAGGTITT |
| SLCC2A3 U-R | Human | ACTCTAAAACCCAAATCTCTCAACA |

F, forward; R, reverse; M, methylated; U, unmethylated; SLC22A3, solute carrier family 22 member 3.
according to the manufacturer’s protocol. DNA bisulfite transformation, purification, and detection of DNA concentration and purity were conducted using a DNA bisulfite transformation kit (centrifugal column type) [cat. no. DP215; Tiangen Biotech (Beijing) Co., Ltd.] according to the manufacturer’s protocol. Subsequently, MSP was performed using a MSP kit [cat. no. EM101; Tiangen Biotech (Beijing) Co., Ltd.], according to manufacturer’s protocol. After the reaction, 10 µl product was obtained for agarose gel electrophoresis (12% agarose) and the products of gel electrophoresis were retrieved according to E.Z.N.A.® Gel Extraction kit (cat. no. D2500-01; Omega Bio-Tek, Inc.). The PCR product was linked to the pClone007 vector using the pClone007 vector kit (cat. no. TSV-007; Beijing TsingKe Biotech Co., Ltd.) and identified through sequencing by TSINGKE Biological Technology.

Xenograft tumor formation assay in nude mice. Animal experiments were approved by the Ethical Committee for Animal Research of Sichuan University. HCT116 cells (5x10³ cells; 100 µl) were subcutaneously inoculated into the upper right flank of 6-week-old male nude mice (total n=40; weight, ~15 g). BALB/c- nu/nu mice were purchased from Chengdu Dossy Experimental Animals Co., Ltd. The mice were housed under a 12-h light/dark cycle at 25°C with 50-60% humidity and free access to food and water. The length and width of tumors were measured using calipers and the tumor volume was calculated as follows: \( V = \frac{(length \times width)^2}{2} \) twice per week. The relative tumor volume (RTV) was calculated as follows: \( RTV = \frac{V}{V_0} \). Where \( V_0 \) is the tumor volume at grouping and \( V_t \) is the tumor volume at each measurement. Approximately 10 days following inoculation, nude mice with tumor volumes of 100-200 mm³ were selected and divided into groups as follows: Control group, DAC group, OXA group and combination group. DAC (2.5 mg/kg) was intraperitoneally administered into mice in the DAC and combination groups every 3 h on days 1, 11 and 21, three times a day. OXA (10 mg/kg) was intraperitoneally administered once into mice in the OXA and combination groups on days 8, 13, 18, 23, 28 and 33. Mice in the control group were intraperitoneally administered an equal volume of solvent (5% glucose). DAC, decitabine; OXA, oxaliplatin.

Results

Induction of OCT3 expression by DAC. According to our preliminary experiments, HCT116 and SW620 cells were treated with <50 µM DAC, and HT29 cells were treated with <100 µM DAC, at which cell viability was >80%, in order to detect the effects of DAC on OCT3 expression in the three CRC cell lines. Baseline SLC22A3 mRNA expression was lowest in HCT116 cells among the three CRC cell lines, and the mRNA expression levels of SLC22A3 were significantly higher in HT29 and SW620 cells (P<0.05, Fig. 2A). SLC22A3 mRNA expression was significantly induced in HCT116 cells following treatment with DAC for 72 h in a concentration-dependent manner (Fig. 2B), whereas SLC22A3 expression was not induced by DAC in SW620 and HT29 cells (Fig. 2C and D). The three CRC cell lines were then treated with 2.5 µM DAC; the results revealed that the induction of SLC22A3 mRNA expression was time-dependent in HCT116 cells, but not in HT29 or SW620 cells (Fig. 2E-G). The induction of OCT3 protein expression by DAC was similar to that of its mRNA expression. DAC could upregulate OCT3 expression in HCT116 cells, in which the baseline expression of OCT3 was low, in a concentration- and time-dependent manner, but not in SW620 or HT29 cells, in which the baseline expression levels of OCT3 were high (Fig. 2H-J).

Methylation status of the SLC22A3 gene promoter region as determined by MSP in CRC cells. To investigate the methylation status of the SLC22A3 gene promoter region in CRC cells, MSP was conducted. Methylation (-) and non-methylated products (+) in SW620 and HT29 cells, and methylated (+) and non-methylated products (-) in HCT116 cells (Fig. 3A). As shown in Fig. 3B, methylation products decreased and non-methylation products increased in HCT116 cells treated with 2.5 µM DAC for 48 and 72 h, respectively. Sequencing revealed that the methylated PCR products were consistent with the target sequence (Fig. 3C). These findings confirmed that the methylation products were the desired products.

Influence of DAC on the effects of OXA and OCT3 on CRC cells. To explore the influence of DAC on the inhibitory effects of OXA on CRC cells, CRC cell lines were treated with OXA following treatment with 2.5 µM DAC for the indicated durations.
The results demonstrated that the concentration-activity curves of OXA declined after HCT116 cells were treated with 2.5 µM DAC for 48 and 72 h, but not for 24 h (Fig. 4A), and the IC₅₀ values were also decreased (Table III). There was no significant effect on SW620 cells treated with 2.5 µM DAC for 48 or 72 h, nor HT29 cells for 72 h (Fig. 4B and C). Compared
with wild-type HCT116 cells, the concentration-activity curve of OXA in HCT116 cells treated with sh-OCT3 shifted to the right after treatment with DAC (Fig. 4D). The IC$_{50}$ value of OXA was markedly higher in HCT116 cells expressing sh-OCT3 compared with in wild-type HCT116 cells following treatment with DAC (Table III). Furthermore, the effects of OCT3 on the concentration of OXA in HT29 cells were determined; OXA concentration in HT29 cells with SLC22A3 knockdown (sh-OCT3) was significantly lower than that in the control group (Fig. 4E). The knockdown efficiency on SLC22A3 in HCT116 and HT29 was both more than 75%.

**Table III. Effects of DAC on IC$_{50}$ of OXA in colorectal cancer cells.**

| Cell line     | 0 h (95% CI)     | 24 h (95% CI)    | 48 h (95% CI)    | 72 h (95% CI)    |
|---------------|------------------|------------------|------------------|------------------|
| HCT116        | 8.64 (3.68-20.29)| 8.37 (3.11-22.47)| 2.38 (0.70-8.1)  | 1.50 (0.62-3.62) |
| SW620         | 71.94 (56.92-90.91) | 70.22 (63.78-77.33) | 74.41 (64.1-86.37) | 42.89 (31.10-59.16) |
| HT29          | 37.58 (23.76-59.43) |                    |                  |                  |
| HCT116-sh-OCT3| 76.61 (55.59-105.6) |                  |                  |                  |

Data are presented as the mean (95% CI). sh, short hairpin RNA; OCT3, organic cation transporter 3.

**Figure 4. Impact of DAC on the inhibitory effects of OXA and OXA concentrations in colon cancer cells.** Alterations in the concentration-activity curves of OXA in colon cancer cells following treatment with 2.5 µM DAC for (A) 0, 24, 48 and 72 h in HCT116 cells, (B) 0, 48 and 72 h in SW620 cells and (C) 0 and 72 h in HT29 cells. Impact of solute carrier family 22 member 3 knockdown (sh-OCT3) on (D) inhibitory effect of OXA on HCT116 cells and (E) OXA concentrations in HT29 cells. (F) Knockdown efficiency on SLC22A3 in HCT116 and HT29 are both more than 75%. *P<0.05 compared with Control. DAC, decitabine; OCT3, organic cation transporter 3; OXA, oxaliplatin; sh, short hairpin RNA; SLC22A3, solute carrier family 22 member 3.

**Direct effect of OCT3 expression on the migration and invasion of CRC cells.** To explore the role of OCT3 in the migration of CRC cells, wound healing assays were conducted. The results revealed that the percentage of migrating HT29 cells with SLC22A3 knockdown (sh-OCT3) was significantly higher compared with in the control group (Fig. 5A-a and A-b). To investigate the role of OCT3 in CRC cell invasion, the mRNA expression levels of MMP2 were detected; MMP2 is an indicator of cancer cell invasion, whereby elevated MMP2 expression indicates increased invasion (23,24). The results demonstrated that the expression levels of MMP2 were significantly higher in HT29 cells with SLC22A3 knockdown compared with in the control group (Fig. 5B). Consistent with this, MMP2 mRNA expression in HCT116 cells was reduced in a concentration-dependent manner once OCT3 expression was induced by 1.25 and 2.5 µM DAC (Fig. 5C).

**Impact of DAC on the effects of OXA on CRC xenografts and the association with OCT3 expression.** Representative images of xenografts in nude mice are shown in Fig. 6A.
From alterations in RTV curve of xenografts in nude mice, it was revealed that RTV was lowest in the combined DAC and OXA group (Fig. 6B). The inhibitory effect was as follows: Combination group > DAC group > OXA group (Fig. 6A and B). On day 7 following initiation of DAC administration, OCT3 expression in xenografts of mice administered DAC was higher than in xenografts of mice that did not receive DAC (Fig. 7A-a and A-b). At the end of the experiment, OCT3 expression in xenografts of mice administered DAC remained higher than in xenografts of mice that did not receive DAC (Fig. 7B-a and 7B-b). Furthermore, the concentration of OXA in xenografts of mice treated with DAC was higher than in xenografts of mice not treated with DAC (Fig. 7C).

Discussion

This study demonstrated that the DNA methyltransferase inhibitor DAC could increase the effects of OXA on CRC
Tumor cell migration is one of the critical steps in the invasion and metastasis of malignant tumors. Cell migration assays suggested that OCT3 may inhibit the migration of CRC cells; however, the underlying mechanism requires further investigation. MMP2 belongs to the MMP family, and its expression is associated with the invasion and metastasis of CRC (31,32). Therefore, MMP2 was selected as a marker to indicate the possible role of OCT3 in the biological behavior of malignant tumors by detecting the impact of OCT3 expression on MMP2 expression. The results revealed that MMP2 expression was upregulated in SLC22A3-knockdown CRC cells, which indicated that OCT3 may inhibit the invasion and metastasis of CRC by reducing MMP2 expression. However, the mechanism by which OCT3 inhibits MMP2, directly or indirectly, is unclear. Consistent with the present results, Fu et al (12) recently revealed that the expression of SLC22A3 in non-tumorous tissues of patients with familial esophageal cancer is significantly downregulated, and adenosine-to-inosine RNA editing of this gene leads to downregulation of its expression and is significantly associated with lymph node metastasis. Further investigations have reported that OCT3 can directly bind α-actinin-4 (ACTN4), and may inhibit ACTN4-mediated actin cross-linking and cell migration (12). A limitation of the present study is that, for migration assays, 1% serum-containing medium was added.
to the culture dish following wound generation, instead of serum-free medium. Although 1% serum-containing medium has little effect on cell proliferation, it may not completely exclude the effect of cell proliferation on the experiment.

In conclusion, OCT3 may directly inhibit the malignant biological behavior of cancer and may be considered a novel target for intervention in CRC. OCT3-specific inhibitors have recently been reported (33,34), and will be useful tools for further investigating the function of OCT3.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors’ contributions
JG, XJ and LW were involved in conception and design. JG and WZ conducted cell biological experiments and xenograft experiments. JG and TL analyzed and interpreted the data. JG, ST and WZ conducted cell biological experiments and xenograft experiments. JG, ST and WZ conducted cell biological experiments and xenograft experiments. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Animal experiments were approved by the Ethical Committee for Animal Research of Sichuan University.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Perego P and Robert J: Oxaliplatin in the era of personalized medicine: From mechanistic studies to clinical efficacy. Cancer Chemother Pharmacol 77: 5-18, 2016.
2. Martínez-Balibrea E, Martínez-Cardús A, Gines A, Ruiz-de Porras V, Moutinho C, Layos L, Manzano JL, Bugés C, Bystrup S, Esteller M and Abad A: tumor-related molecular mechanisms of oxaliplatin resistance. Mol Cancer Ther 14: 1767-1776, 2015.
3. Buss I, Garmann D, Galanski M, Weber G, Kalayda GV, Keppeler BK and Jaeckle U: Enhancing lipophilicity as a strategy to overcome resistance against platinum complexes. J Inorg Biochem 105: 709-717, 2011.
4. Oguri T, Kunii E, Fukuda S, Sone K, Uemura T, Takakura O, Kanemitsu Y, Ohkubo H, Takemura M, Maeno K, et al: Organic cation transporter 6 directly confers resistance to anticancer platinum drugs. Biochem Biophys Res Commun 38: 639-643, 2016.
5. Wu X, Kekuda R, Huang W, Fei YJ, Leibach FH, Chen J, Conway SJ and Ganapathy V: Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. J Biol Chem 273: 32776-32786, 1998.
6. Koepsell H: Polyspecific organic cation transporters: Their functions and interactions with drugs. Trends Pharmacol Sci 25: 375-381, 2004.
7. Yokoo S, Masuda S, Yonezawa A, Terada T, Katsura T and Inui K: Significance of organic cation transporter 3 (SLC22A3) expression for the cytotoxic effect of oxaliplatin in colorectal cancer. Drug Metab Dispos 36: 2299-2306, 2008.
8. Zhang S, Lovejoy KS, Shima JE, Lagacac LN, Shu Y, Lapuk A, Chen Y, Komori T, Gray JW, Chen X, et al: Organic cation transporters are determinants of oxaliplatin cytotoxicity. Cancer Res 66: 8847-8857, 2006.
9. Grisanzio C, Werner L, Takeda D, Awoyemi BC Pomerantz MM, Yamada H, Sooriakumaran P, Robinson BD Leung R, Schinzel AC, et al: Genetic and functional analyses implicate the NUDT11, HNF1B, and SLC22A3 genes in prostate cancer pathogenesis. Proc Natl Acad Sci USA 109: 11252-11257, 2012.
10. Vollmar J, Lautem A, Closs E, Schuppan D, Kim YO, Grimm D, Marquardt JU, Fuchs P, Straub BK, Schad A, et al: Loss of organic cation transporter 3 (Oct3) leads to enhanced proliferation and hepatocarcinogenesis. Oncotarget 8: 115667-115680, 2017.
11. Xiong JX, Wang YS, Sheng J, Xiang D, Huang TX, Tan BB, Zeng CM, Li HH, Yang J, Meltzer SJ, et al: Epigenetic alterations of a novel antioxidant gene SLC22A3 predispose susceptible individuals to increased risk of esophageal cancer. Int J Biol Sci 14: 1668-1668, 2018.
12. Wu F, Qin YR, Ming XY, Zuo XB, Diao YW, Zhang LY, Ai J, Liu BL, Huang TX, Cao TT, et al: RNA editing of SLC22A3 drives early tumor invasion and metastasis in familial esophageal cancer. Proc Natl Acad Sci USA 114: E4631-E4640, 2017.
13. Guo C, Ma J, Deng G, Qu Y, Yin L, Li Y, Han Y, Cai C, Chen H and Zeng S: ZEB1 promotes oxaliplatin resistance through the induction of epithelial-mesenchymal transition in colon cancer cells. J Cancer 8: 3555-3566, 2017.
14. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA Cancer J Clin 63: 11-30, 2013.
15. Kaiser C, Meurice N, Gonzales IM, Arora S, Beaudry C, Bisanz KM, Robeson AC, Petit J and Azorsa DO: Chemogenomic analysis identifies Mabecin II as a compound specific for SMAD4-negative colon cancer cells. Chem Biol Drug Dis 75: 360-368, 2010.
16. Luebeck GE, Hazeldon WD, Curtis K, Maden SK, Yu M, Carter KT, Burke W, Lampe PD, Li CI, Ulrich CM, et al: Implications of epigenetic drift in colorectal neoplasia. Cancer Res 79: 495-504, 2019.
17. Zhou C, Pan R, Hu H, Li B, Dai J, Ying X, Yu H, Zhong J, Mao Y, Zhang Y, et al: TNFRSF10C methylation is a new epigenetic biomarker for colorectal cancer. PeerJ 6: e5336, 2018.
18. Chu CH, Chang SC, Wang HH, Yang SH, Lai KC and Lee TC: Prognostic values of EPDR1 hypermethylation and its inhibitory function on tumor invasion in colorectal cancer. Cancers (Basel) 10: E393, 2018.
19. Yokoi K, Harada H, Yokota K, Ishii S, Tanaka T, Nishizawa N, Shimazu M, Kojo K, Miura H, Yamasashi T, et al: Epigenetic status of CDO1 gene may reflect chemosensitivity in colon cancer with postoperative adjuvant chemotherapy. Ann Surg Oncol 26: 406-414, 2018.
20. Li J and Dahiya R: MethPrimer: Designing primers for methylation analysis and interactions with drugs. Trends Pharmacol Sci 25: 375-381, 2004.
21. Oguri T, Kunii E, Fukuda S, Sone K, Uemura T, Takakura O, Kanemitsu Y, Ohkubo H, Takemura M, Maeno K, et al: Organic cation transporter 6 directly confers resistance to anticancer platinum drugs. Biomed Rep 5: 639-643, 2016.
22. Wu X, Kekuda R, Huang W, Fei YJ, Leibach FH, Chen J, Conway SJ and Ganapathy V: Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. J Biol Chem 273: 32776-32786, 1998.
24. Chen Z, Ji N, Wang Z, Wu C, Sun Z, Li Y, Hu F, Wang Z, Huang M and Zhang M: Fine particulate matter (PM$_{2.5}$) promoted the invasion of lung cancer cells via an ARNT2/PP2A/STAT3/MMP2 pathway. J Biomed Nanotechnol 15: 416, 2019.

25. Shnitsar V, Eckardt R, Gupta S, Grottker J, Müller GA, Koepsell H, Burckhardt G and Hagos Y: Expression of human organic cation transporter 3 in kidney carcinoma cell lines increases chemosensitivity to melphalan, irinotecan, and vincristine. Cancer Res 69: 1494-1501, 2009.

26. Hsu CM, Lin PM, Chang JG, Lin HC, Li SH, Lin SF and Yang MY: Upregulated SLC22A3 has a potential for improving survival of patients with head and neck squamous cell carcinoma receiving cisplatin treatment. Oncotarget 8: 74348-74358, 2017.

27. Hagiwara H, Sato H, Ohde Y, Takano Y, Seki T, Ariga T, Hokiawado N, Asamoto M, Shirai T, Nagashima Y and Yano T: 5-Aza-2'-deoxycytidine suppresses human renal carcinoma cell growth in a xenograft model via up-regulation of the connexin 32 gene. Br J Pharmacol 153: 1373-1381, 2008.

28. Qin T, Jelinek J, Si J, Shu J and Issa JP: Mechanisms of resistance to 5-aza-2'-deoxycytidine in human cancer cell lines. Blood 113: 659-667, 2009.

29. Hosokawa M, Saito M, Nakano A, Iwashita S, Ishizaka A, Ueda K and Iwakawa S: Acquired resistance to decitabine and cross-resistance to gemicitabine during the long-term treatment of human HCT116 colorectal cancer cells with decitabine. Oncol Lett 10: 761-767, 2015.

30. Zhao H, Zhu H, Huang J, Zhu Y, Hong M, Zhu H, Zhang J, Li S, Yang L, Lian Y, et al: The synergy of vitamin C with decitabine activates TET2 in leukemic cells and significantly improves overall survival in elderly patients with acute myeloid leukemia. Leuk Res 66: 1-7, 2018.

31. Egeblad M and Werb Z: New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2: 161-174, 2002.

32. Zhu XH, Wang JM, Yang SS, Wang FF, Hu JL, Xin SN, Men H, Lu GF, Lan XL, Zhang D, et al: Down-regulation of DAB2IP promotes colorectal cancer invasion and metastasis by translocating hnRNPK into nucleus to enhance the transcription of MMP2. Int J Cancer 141: 172-183, 2017.

33. Hu T, Wang L, Pan XL and Qi HL: Novel compound, organic cation transporter 3 detection agent and organic cation transporter 3 activity inhibitor, WO2015002150 A1: A patent evaluation. Expert Opin Ther Pat 26: 857-860, 2016.

34. Pan X, Iyer KA, Liu H, Sweet DH and Dukat M: A new chemotype inhibitor for the human organic cation transporter 3 (hOCT3). Bioorg Med Chem Lett 27: 4440-4445, 2017.

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