Neoplasma 2022; 69(4): 776–784

doi:10.4149/neo_2022_211230N1854

Solute carrier organic anion transporter family member 4A1 promotes colorectal cancer progression and is regulated by miR-1224-5p

Yan JIANG[1], Xizhen ZHOU[2], Yingying LI[3], Xiaojie GAO[3,*]

[1]Department of TCM Pharmacy, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; [2]Department of Traditional Chinese Medicine, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; [3]Department of Pharmacy, The Second Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China

**Correspondence: gaoxiaojie83@163.com*

Received December 30, 2021 / Accepted March 14, 2022

Colorectal cancer (CRC) is one of the most common malignant tumors, and pharmacological treatments of CRC are unsatisfactory. Increasing evidence shows that solute carrier organic anion transporter family member 4A1 (SLCO4A1) is abnormally expressed in numerous cancer types and may be correlated with cancer development and metastasis. However, the roles of SLCO4A1 in CRC are incompletely understood. This study utilized the GSE110224 dataset and other databases to analyze SLCO4A1 expression levels in CRC tissues. The expression levels of SLCO4A1 in CRC cell lines were evaluated by quantitative real-time polymerase chain reaction and western blotting. The roles of SLCO4A1 in CRC cell proliferation, migration, invasion, and epithelial-mesenchymal transition were assessed. The interaction between SLCO4A1 and microRNA-1224-5p was verified using a dual-luciferase reporter assay. The effect of SLCO4A1 *in vivo* was investigated using a BALB/c mouse model. The level of SLCO4A1 expression was increased in CRC tissues and cell lines. Moreover, high SLCO4A1 expression was positively associated with a poor prognosis. The results of gain- and loss-of-function experiments showed that SLCO4A1 knockdown suppressed CRC cell proliferation, migration, invasion, and epithelial-mesenchymal transition while SLCO4A1 overexpression had opposite effects *in vitro*. Furthermore, SLCO4A1 knockdown could suppress tumor growth *in vivo*. Further analyses showed that SLCO4A1 was downregulated by miR-1224-5p. Rescue experiments confirmed that SLCO4A1 reversed the effect of miR-1224-5p on cell function. These results suggested that SLCO4A1 acted as an oncogene to regulate CRC development and was a potential target for CRC treatment.

Key words: colorectal cancer, SLCO4A1, miR-1224-5p

Colorectal cancer (CRC) is one of the most frequent malignancies and the fourth leading cause of morbidity worldwide [1]. Furthermore, despite advances in radical surgery and neoadjuvant therapy, the 5-year survival rate of CRC patients is low [2, 3]. Thus, a better understanding of the molecular mechanisms underlying CRC tumorigenesis is essential to improve the prognosis of CRC.

Solute carrier organic anion transporter family member 4A1 (SLCO4A1) (also known as OATP4A1), belonging to the solute carrier organic anion transporter family (SLCO), plays important role in transporting proteins, and endogenous and exogenous compounds [4]. SLCO4A1 is abnormally expressed in numerous cancer types and may be correlated with cancer development and metastasis [5–7]. Recently, Wu et al. propose that inhibition of SLCO4A1 expression by downregulation of SLCO4A1-AS1 protects against CRC progression [8]. However, the biological roles of SLCO4A1 in CRC are incompletely understood.

microRNAs (miRNAs) are short-chain non-coding RNA transcripts that directly bind to the 3' untranslated region of target messenger RNAs to inhibit gene expression [9]. miRNAs regulate cancer cell proliferation, migration, invasion, and apoptosis [10]. For instance, miR-1258 inhibits CRC proliferation by directly targeting E2F8 [11]. miR-375 enhances 5-fluorouracil chemosensitivity by targeting thymidylate synthase in CRC [12], and miR-150-5p suppresses CRC progression by targeting VEGFA [13]. Therefore, understanding the function of miRNAs is essential to improve the diagnosis and treatment of CRC.

This study found that SLCO4A1 was highly expressed in CRC tissues and cell lines, and SLCO4A1 was positively correlated with the poor prognosis of CRC. Functional experiments were performed to assess the function of SLCO4A1 *in vitro* and *in vivo*. Furthermore, SLCO4A1 was negatively regulated by miR-1224-5p. These results demonstrated that SLCO4A1 was a potential target for CRC treatment.

Materials and methods

Materials. RPMI-1640, fetal bovine serum (FBS), Lipofectamine® 2000 transfection reagent, Click-iT EdU Imaging Kit, TRIzol® reagent, and SLCO4A1 antibody (1:1000, A304-457A) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Small interfering RNAs (siRNAs) targeting SLCO4A1 (si-SLCO4A1) and a SLCO4A1 overexpression vector (ov-SLCO4A1) were synthesized by GenePharma (Shanghai, China). miR-1224-5p mimics and inhibitors were synthesized by RiboBio (RiboBio, Guangzhou, China). Primary antibodies against E-cadherin, vimentin, N-cadherin, and GAPDH were purchased from Cell Signaling Technology, Inc. (CST) (Danvers, MA, USA).

Cell culture and transfection. Human CRC cell lines LoVo and HT29 and normal colonic epithelial cells NCM460 were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI-1640 medium supplemented with 10% FBS in a humidified incubator at 37 °C with 5% CO_2.

For transfection, 1 ml of LoVo and HT29 cells (2×10^5/ml) was seeded in each well of a 6-well plate. After reaching 70–80% confluence, cells were transfected with oligonucleotides (miR-1224-5p mimics and inhibitors at a final concentration of 5 μM) using Lipofectamine® 2000 following the manufacturer's protocol. After 6 h of incubation at 37 °C, the transfection mixture was replaced with a fresh medium, and cells were cultured for another 48 h.

Measurement of mRNA expression by quantitative real-time PCR (qRT-PCR). Total RNA from transfected CRC cells was extracted using TRIzol® reagent. Then the extracted RNAs were reverse transcribed into cDNA by PrimeScript™ RT reagent kit (Takara, Dalian, China). The miRNA expression levels were analyzed using a Mir-X miRNA First-Strand Synthesis Kit (Takara). The expression levels of miR-1224 and SLCOA41 were measured by qRT-PCR on an ABI 7500 Fast system (Applied Biosystems, Waltham, MA, USA). The transcription level of each target gene was normalized to the housekeeping gene U6 or GAPDH using the $2^{-\Delta\Delta Ct}$ method.

Western blotting. Total protein was extracted using RIPA lysis buffer (Beyotime, Guangzhou, China), and protein concentration was measured using the BCA Protein Assay Kit. Proteins (40 μg in each line) from each cell line were separated by SDS-PAGE and transferred to PVDF membranes. The membranes were blocked in 5% non-fat milk in TBST for 2 h at room temperature and incubated with primary antibodies at 4 °C overnight. Then, the membranes were incubated with HRP-conjugated secondary antibodies for 2 h. Immunoreactive bands were visualized using an enhanced chemiluminescence detection system. Protein expression was normalized to GAPDH expression levels. The antibodies, including Vimentin (#5741), N-Cadherin (#13116), E-Cadherin (#14472), GAPDH (#5174), anti-rabbit IgG (#7074), and anti-mouse IgG (#7076) were all purchased CST (Danvers, MA, USA). SLCO4A1 antibody (A304-457A) was purchased from Thermo Fisher Scientific (Shanghai, China).

Wound-healing assay. Transfected CRC cells (5×10^5 cells/well) were seeded on six-well plates. Upon reaching full confluence, the cell monolayer was scratched using a sterile 10 μl pipette tip. Then, cells were washed with sterile PBS three times and cultured in a fresh serum-free medium. At 0 and 24 h after scratch, cells were observed under a light microscope, and relative cell migration was quantified.

Transwell migration assay. The invasion of CRC cells was assessed using a 24-well Transwell system (BD Bioscience, San Jose, CA, USA). Briefly, Matrigel (1:8 dilution; BD Biosciences) was added to the upper compartment of a Transwell chamber and solidified at 37 °C. Transfected cells (5×10^4) were resuspended in 600 μl of RPMI-1640 medium supplemented with 0.1% FBS and plated on the Matrigel-coated upper chamber, while 600 μl of culture medium supplemented with 20% FBS was added to the lower chamber. The cells were cultured in an incubator at 37 °C for 48 h. The cells in the upper chamber were removed with cotton swabs and washed with PBS three times. The cells that crossed the Transwell membrane were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet for 30 min at room temperature. Cells were observed under a light microscope at 200× magnification (Olympus Corporation, Tokyo, Japan) and counted in five randomly selected fields.

Cell counting kit-8 (CCK-8) assay. Cell viability was measured using a CCK-8 kit according to the manufacturer's instructions. Briefly, cells in the logarithmic growth phase were cultured in a serum-free medium for 24 h, digested, diluted to a concentration of 5×10^4/ml, seeded in 96-well plates (100 μl), and cultured in a humidified incubator with 5% CO_2 at 37 °C. After 24, 48, and 72 h, 100 μl of the medium was replaced with the same volume of a fresh medium, and 10 μl of CCK-8 reagent was added to each well. The plates were incubated for 2 h in the dark, and cell viability was assessed by measuring absorbance at 450 nm using a microplate reader.

EdU assay. Transfected cells were seeded in 96-well plates ($2–3\times10^4$ cells/ml) and cultured in a humidified incubator with 5% CO_2 at 37 °C overnight. Then, cells were incubated with EdU (10 μM) for 2 h, harvested, fixed with 4% paraformaldehyde at room temperature for 15–30 min, and permeabilized with PBS containing 0.5% Triton X-100 for 20 min. After washing with PBS, samples were incubated with 100 μl of reaction buffer in the dark at room temperature for 30 min. Nuclei were stained with Hoechst 33342. Cells were imaged on an inverted fluorescence microscope.

Luciferase reporter assay. Putative binding sites between the SLCO4A1 3'-UTR and miR-1224-5p seed sequence were predicted by TargetScan (www.targetscan.org). The wild-type or mutant 3'-UTR of SLCO4A1 harboring miR-1224-5p binding sites was constructed by GenePharma. Then, 293T cells were co-transfected with plasmids and miR-1224-5p mimic or the negative control and seeded in 24-well plates.

After 48 h of transfection, luciferase activity was detected using the Dual-Luciferase Reporter Assay Kit (Promega, Madison, WI, USA). The binding between SLCO4A1 and miR-1224-5p was assessed by calculating the ratio of Renilla luciferase to firefly luciferase activity.

Immunofluorescence staining. LoVo and HT29 cells were fixed with 4% paraformaldehyde for 15 min at room temperature and blocked with 5% BSA for 30 min. Cells were washed with PBS and incubated with anti-vimentin antibody (#5741, CST) at 4 °C overnight and with secondary antibody (#4413, CST) at 4 °C for 2 h. Nuclei were counter-stained with 0.1% DAPI for 5 min. Cells were imaged on a fluorescence microscope.

Animal studies. Briefly, 4-week-old BALB/c nude mice (Gem Pharmatech; Nanjing, China) were randomly divided into two groups (n=6 in each group). LoVo cells were transduced with lentivirus expressing SLCO4A1 (sh-SLCO4A1) or a negative control lentivirus (sh-NC). Cells (2×10^6) were suspended in 100 µl of PBS and injected subcutaneously into the lateral flank of mice to form tumors. Tumor size was measured every 2 days using a Vernier caliper. When the tumor volume of the sh-NC group reached 1000 mm^3, mice were sacrificed, the tumors were surgically dissected, and tumor volume was calculated using the equation V = 0.5 × (length × square width). All animal studies were approved by the Animal Care and Use Committee of Zhejiang Chinese Medicine University and were performed in accordance with institutional guidelines.

Statistical analysis. Statistical analysis was performed using GraphPad Prism version 7.0. Continuous data were analyzed using a one-way analysis of variance or Student's t-test and were expressed as the mean ± standard deviation. p-values of <0.05 were considered statistically significant. All experiments were performed in triplicate and repeated independently at least three times.

Results

SLCO4A1 expression was increased in CRC tissues and cell lines. We first analyzed the GSE110224 dataset and found that SLCO4A1 expression was significantly higher in CRC tissues than in normal tissues (Figure 1A). starBase database (http://starbase.sysu.edu.cn/index.php) is a comprehensive database that provides information on the interaction between miRNA and various RNA molecules [14], and GEPIA (http://gepia.cancer-pku.cn/index.html), designed by Peking University, is a database for analyzing RNA sequence expression [15]. The analyses of the starBase and GEPIA databases confirmed that SLCO4A1 was overexpressed in CRC tissues, indicating that SLCO4A1 was upreg-

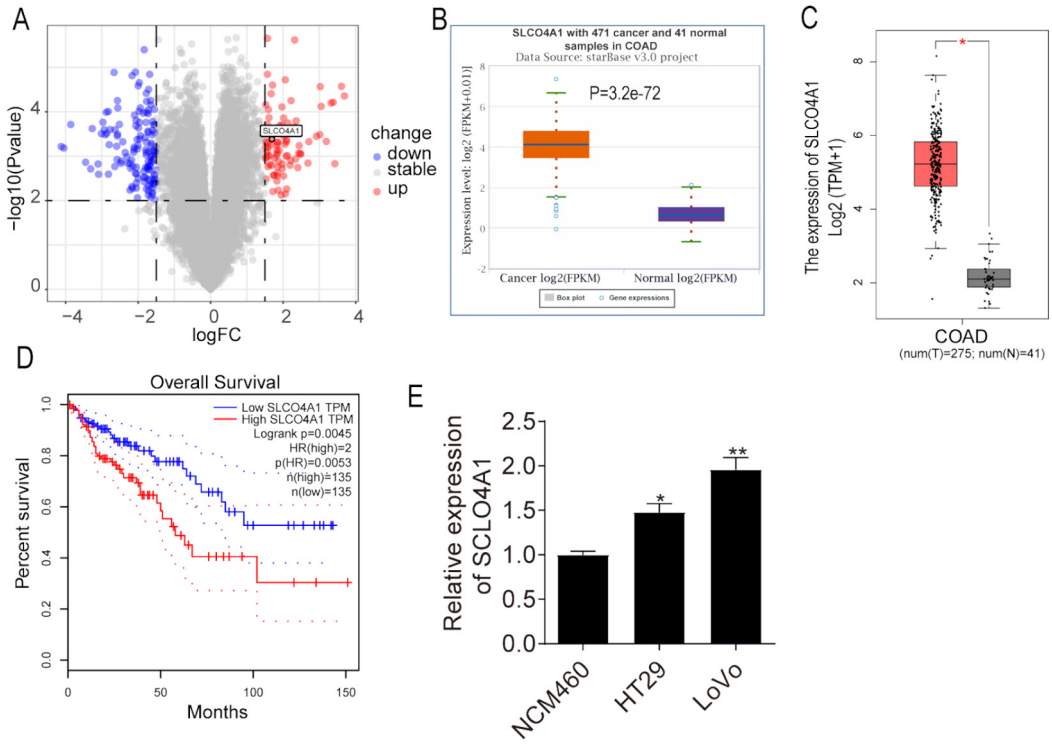

Figure 1. SLCO4A1 expression is increased in colorectal cancer (CRC) tissues and cell lines. A) Expression of mRNAs in CRC and normal tissues. The red and blue dots represent differentially expressed dysregulated mRNAs. Among these, SLCO4A1 expression was increased. The expression of SLCO4A1 in CRC and normal tissues was analyzed using starBase (B) and GEPIA (C) databases. D) The relationship between SLCO4A1 expression and the prognosis of CRC patients was analyzed using the GEPIA database. E) SLCO4A1 expression detected by RT-qPCR was higher in CRC cells than in normal cells. *p<0.05, **p<0.01

ulated in CRC (Figures 1B, 1C). Overall survival was significantly lower in patients with high SLCO4A1 expression than in patients with low SLCO4A1 expression, indicating that SLCO4A1 expression level was correlated with the poor prognosis of CRC patients (p=0.0053, Figure 1D). Moreover, SLCO4A1 expression level was significantly higher in CRC cells than in the normal cell line NCM460 (Figure 1E). These results suggested that SLCO4A1 was involved in CRC development as an oncogene and correlates with the poor prognosis of CRC patients.

SLCO4A1 regulates the proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) of CRC cells *in vitro*. To elucidate the biological function of SLCO4A1 in CRC, SLCO4A1 expression was downregulated in LoVo cells using siRNA targeting SLCO4A1 (si-SLCO4A1), and SLCO4A1 expression was upregu-

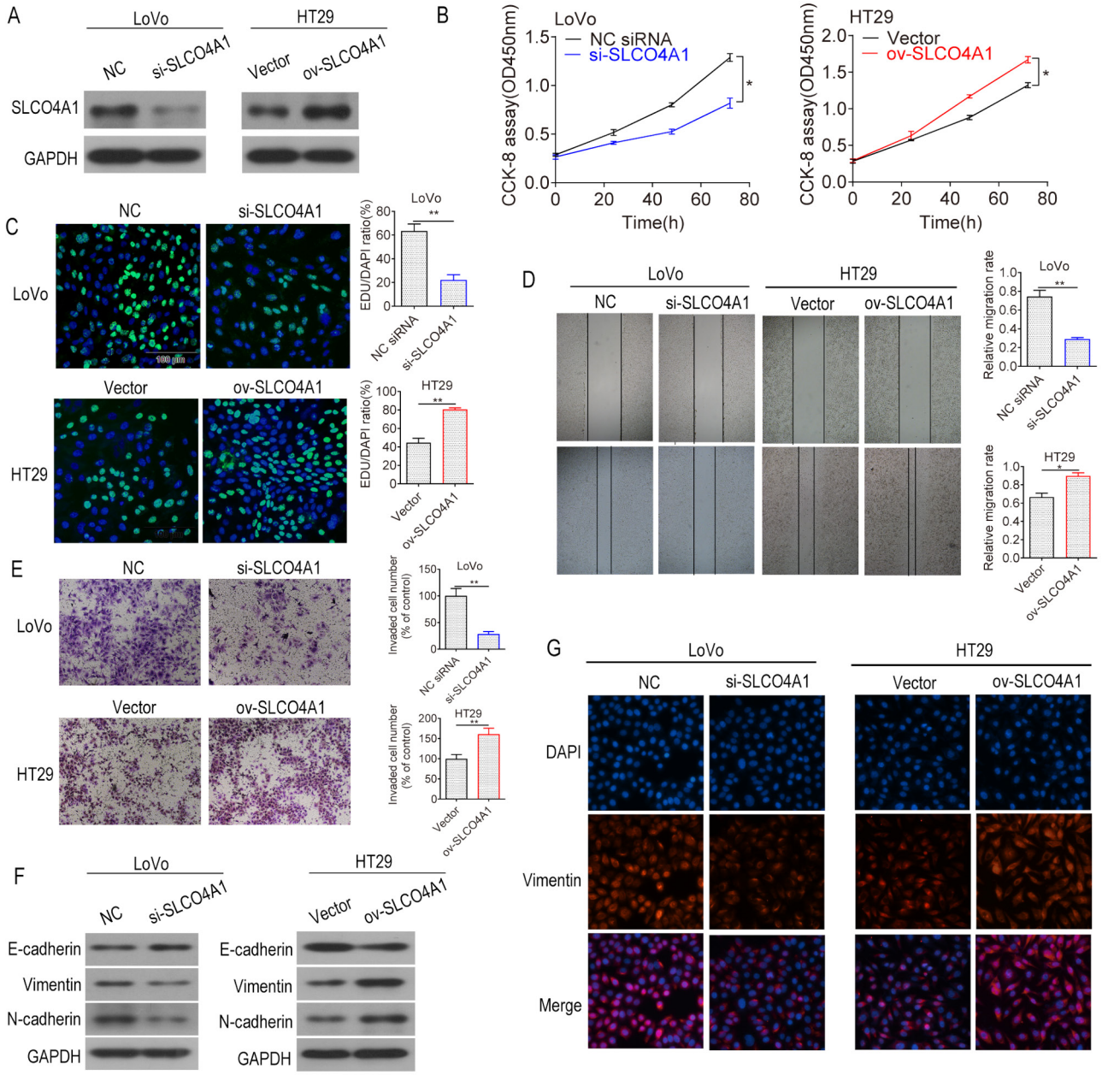

Figure 2. SLCO4A1 promotes the proliferation, migration, invasion, and epithelial-mesenchymal transition of cancer cells *in vitro*. LoVo cells were transfected with SLCO4A1 siRNA, and HT29 cells were transfected with a SLCO4A1-overexpression plasmid. A) The efficiency of SLCO4A1 knockdown or overexpression was evaluated by western blotting. B, C) The proliferation of CRC cells was assessed using CCK-8 and EdU assay. D) The migration of CRC cells was evaluated using a wound-healing assay. E) The invasion of CRC cells was assessed using a Transwell assay. F) The expression levels of E-cadherin, vimentin, and N-cadherin were measured by western blotting. G) Changes in the protein expression of vimentin were assessed by immunofluorescence.

lated in HT29 cells utilizing a SLCO4A1 overexpression plasmid (ov-SLCO4A1). Transfection with si-SLCO4A1 and ov-SLCO4A1 was confirmed by western blotting (Figure 2A). The results of CCK-8, EdU, wound-healing, and Transwell assays demonstrated that SLCO4A1 knockdown significantly inhibited the viability, proliferation, migration, and invasion of LoVo cells (Figures 2B–2E). These assays also revealed that SLCO4A1 overexpression promoted the proliferation, migration, and invasion of HT29 cells (Figures 2B–2E). Furthermore, western blot analysis showed that SLCO4A1 silencing increased E-cadherin levels while decreased N-cadherin and vimentin levels in LoVo cells (Figure 2F). In contrast, SLCO4A1 overexpression had opposite effects on the expression of EMT markers in HT29 cells (Figure 2F). Immunofluorescence showed that SLCO4A1 silencing reduced vimentin expression in LoVo cells, whereas SLCO4A1 overexpression increased vimentin expression in HT29 cells (Figure 2G). These findings demonstrated that SLCO4A1 promoted CRC progression.

SLCO4A1 knockdown inhibits tumor growth *in vivo*. To confirm the effect of SLCO4A1 on CRC tumorigenesis *in vivo*, LoVo cells were transfected with a lentiviral vector expressing an shRNA against SLCO4A1 (sh-SLCO4A1) and corresponding negative control (sh-NC). As shown in Figure 3A, the sizes of tumor tissues in sh-SLCO4A1 groups were smaller than those in NC groups. Consistently, tumor volume and tumor weight were also remarkably reduced by SLCO4A1 knockdown (Figures 3B, 3C). Importantly, we observed decreased Ki-67 expression in the sh-SLCO4A1 groups compared with the NC groups (Figures 3D–3E). These results demonstrated that SLCO4A1 silencing inhibited tumor growth *in vivo*.

SLCO4A1 directly interacts with miR-1224-5p. To explore the underlying mechanisms of *SLCO4A1* in CRC progression, Target Scan (www.targetscan.org) was used to predict the potential miRNAs targeting *SLCO4A1* and miR-1224-5p was predicted to interact with SLCO4A1 (Figure 4A). Luciferase activity assay demonstrated that *SLCO4A1* was a direct target of miR-1224-5p. miR-1224-5p mimic inhibited the luciferase activity of wild-type SLCO4A1-WT but not mutant SLCO4A1 (Figure 4B). The expression levels of miR-1224-5p in CRC tissues were evaluated using starBase. The results showed that the miR-1224-5p expression level was markedly lower in CRC tissues than in normal tissues (Figure 4C). Meanwhile, miR-1224-5p expression was lower in CRC cells than in NCM460 cells (Figure 4D) The mRNA and protein levels of SLCO4A1, measured by qRT-PCR and western blotting, decreased after transfection with miR-1224-5p mimics, whereas the miR-1224-5p inhibitor enhanced SLCO4A1

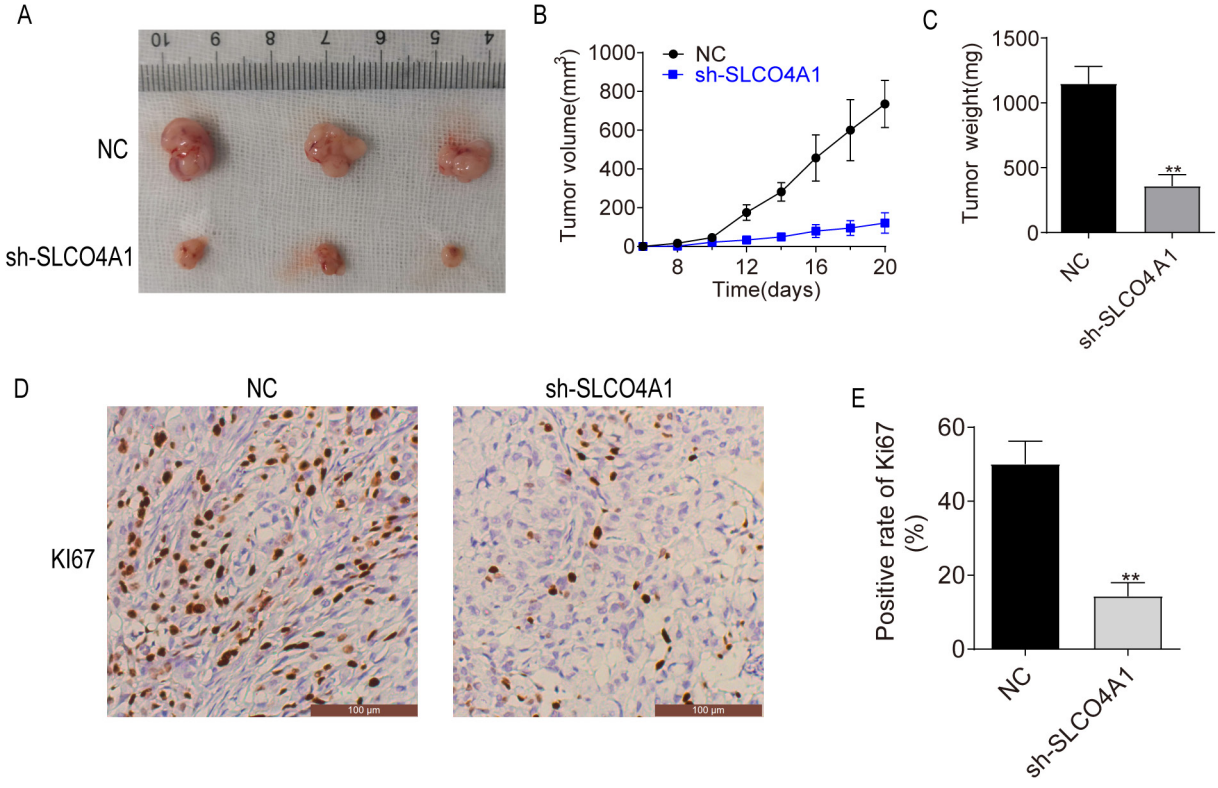

Figure 3. SLCO4A1 knockdown inhibits tumor growth *in vivo*. A) Morphology of colorectal cancer cells transfected with sh-SLCO4A1 or sh-NC. sh-SLCO4A1 decreased tumor volume (B), tumor weight (C), and Ki-67 expression (D–E). **p<0.01

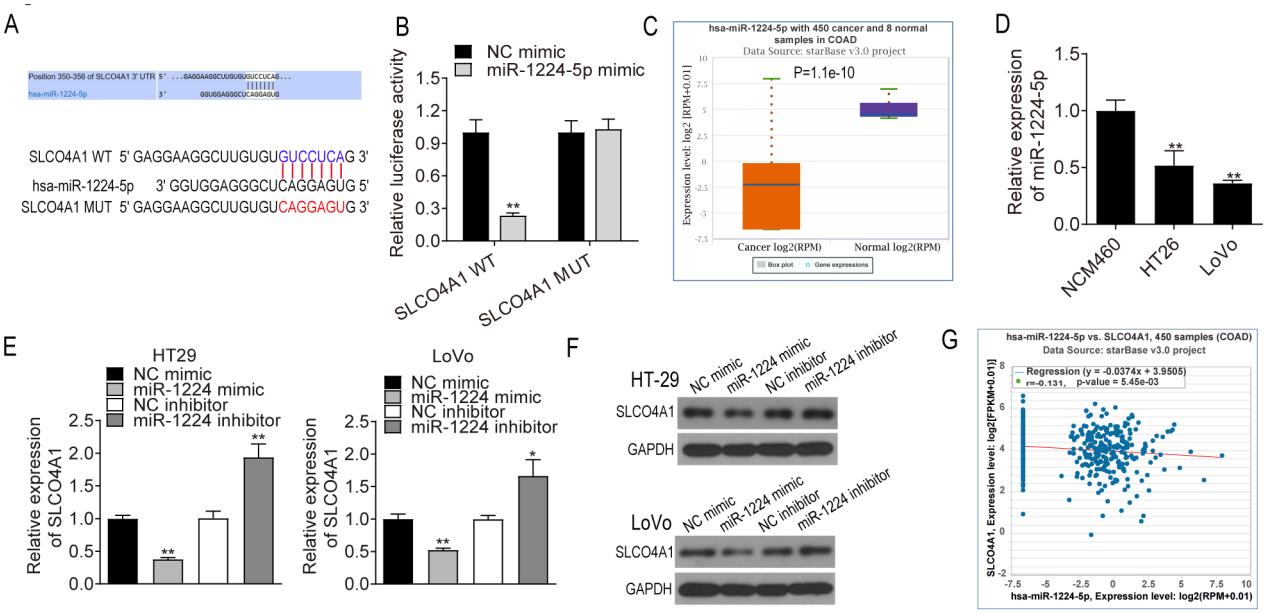

Figure 4. SLCO4A1 directly interacts with miR-1224-5p. A) The interaction between SLCO4A1 and miR-1224-5p was predicted by bioinformatic analysis. B) miR-1224-5p mimic reduced luciferase activity in cells transfected with wild-type SLCO4A1 but not with mutant SLCO4A1. C) The expression of miR-1224-5p in CRC and normal tissues was evaluated using the starBase database. D) miR-1224-5p expression detected by RT-qPCR was lower in CRC cells than in normal cells. E) HT29 and LoVo cells were transfected with miR-1224-5p mimics, miR-1224-5p inhibitor, or the corresponding negative control, and transfection efficiency was investigated by qRT-PCR. F) SLCO4A1 protein levels were assessed by western blotting. G) The interaction between SLCO4A1 and miR-1224-5p was predicted using the starBase database.

expression (Figure 4E, 4F). In addition, the miR-1224 level was inversely correlated with the SLCO4A1 level in CRC tissues (Figure 4G). These results demonstrated that miR-1224-5p directly targeted SLCO4A1.

SLCO4A1 reverses the effects of miR-1224-5p on the proliferation, migration, invasion, and EMT of CRC cells. Next, si-SLCO4A was transfected into miR-1224-5p inhibitor-treated LoVo cells while a SLCO4A1 plasmid was transfected into miR-1224-5p mimic-treated HT29 cells. miR-1224-5p expression levels after transfection were measured by RT-qPCR (Figure 5A). Functional assays revealed that the effects of miR-1224-5p inhibition on the phenotype of LoVo cells were partially reversed by sh-SLCO4A1, whereas the effects of miR-1224-5p mimics on the phenotype of HT29 cells were partially abolished by SLCO4A1 overexpression (Figures 5B–5G). These findings suggested that SLCO4A1 was a downstream target of miR-1224-5p and reversed the effects of miR-1224-5p on CRC cell phenotypes.

Discussion

CRC is the most common gastrointestinal tumor, with high mortality. In 2020, approximately 147,950 people were diagnosed with CRC, of which approximately 53,200 died from the disease [16]. Despite significant advances in the diagnosis and treatment of CRC, the prognosis is poor. Therefore, understanding the mechanisms of CRC progression and identifying novel therapeutic targets for CRC are essential to improve treatment and prognosis.

The solute carrier protein (SLC) family is a class of membrane transporters [17]. SLCs encode organic anion-transporting polypeptides (OATPs) and mediate the sodium-independent transport of endogenous and exogenous compounds, including bile salts, hormones, hormone conjugates, toxins, and drugs [18, 19]. Increasing evidence indicates that OATPs are regulated in several types of cancer and exert a role in cellular processes associated with cancer progression, suggesting a role in the occurrence and development of cancer [20].

The present study analyzed the GSE110224 dataset and found that SLCO4A1 (OATP4A1) expression was higher in CRC tissues than in normal tissues. This result was confirmed by the starBase and GEPIA databases. Furthermore, SLCO4A1 overexpression was associated with the poor prognosis of CRC. In line with these findings, SLCO4A1 expression was shown to be higher in colon cancer and pancreatic adenocarcinoma than in healthy control tissues [8]. These findings suggested that SLCO4A1 functioned as an oncogene in CRC progression.

To further explore the function of SLCO4A1 in CRC progression, si-SLCO4A1 and SLCO4A1 plasmids were transfected into LoVo and HT29 cells, respectively, to assess cell proliferation, migration, and invasion. Gain- and loss-of-function experiments verified that SLCO4A1 knock-

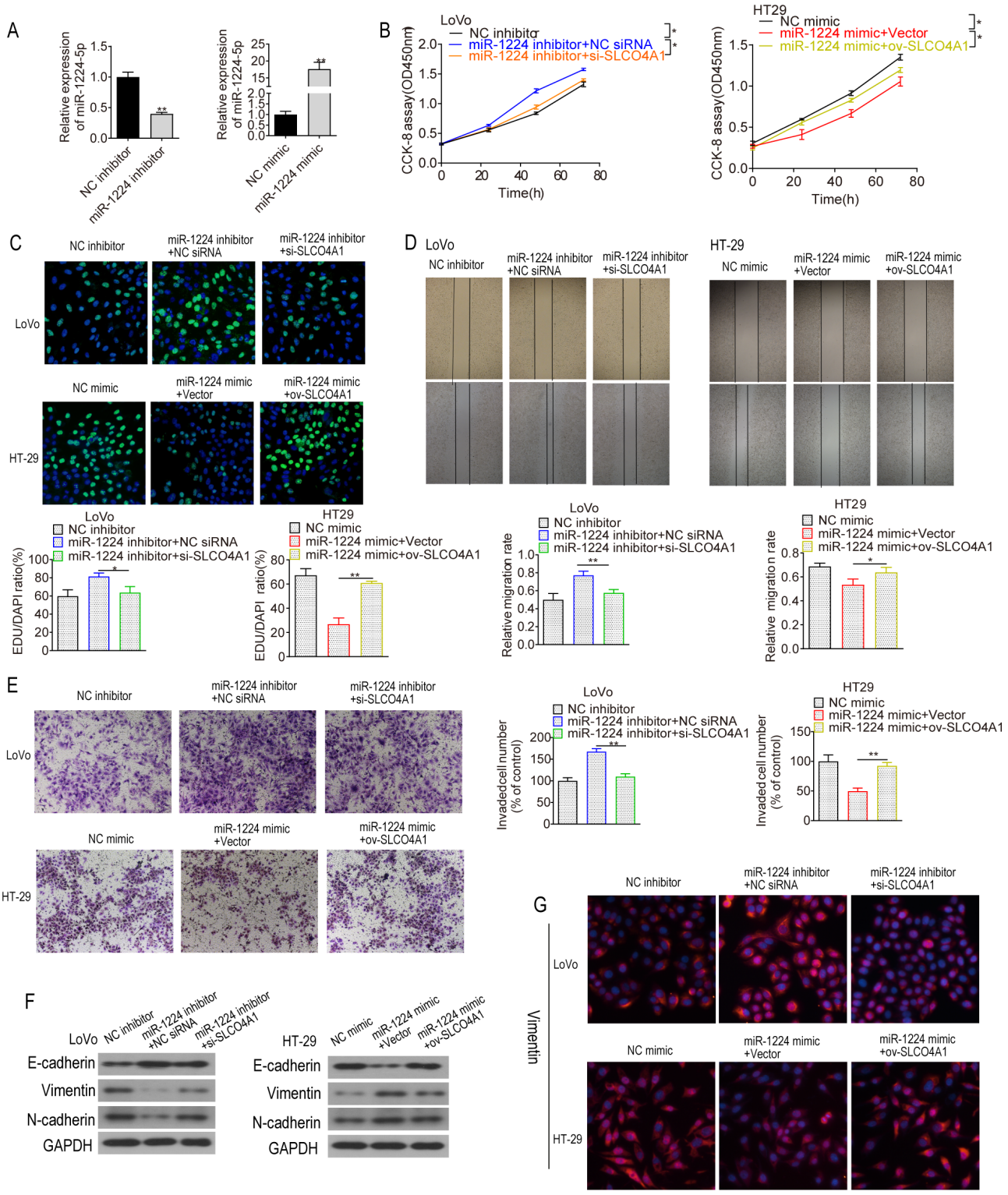

Figure 5. The miR-1224-5p affects the proliferation, migration, invasion, and EMT of colorectal cancer (CRC) cells by regulating SLCO4A1 expression. LoVo cells knocked down for SLCO4A1 were transfected with miR-1224-5p inhibitor, and HT29 cells overexpressing SCLO4A1 were transfected with miR-1224-5p mimics. A) The miR-1224-5p levels in transfected CRC cells were measured by qRT-PCR. B, C) The proliferation of CRC cells after co-transfection was assessed using the CCK-8 and EdU assay. D) The migration of CRC cells after co-transfection was evaluated using a wound-healing assay. E) The invasion of CRC cells after co-transfection was assessed using a Transwell assay. F) The levels of E-cadherin, vimentin, and N-cadherin were determined by western blotting. G) Changes in the protein expression of vimentin were assessed by immunofluorescence.

down suppressed CRC cell proliferation, migration, and invasion *in vitro*. Moreover, *in vivo* experiments demonstrated that SLCO4A1 knockdown inhibited tumor growth, as evidenced by reduced tumor volume, tumor weight, and Ki-67 expression. It has been demonstrated that SLCO4A1 deletion reduces cancer migration, invasion, sphere formation, and tumorigenesis and enhances apoptosis in colon cancer stem cells [8], further supporting our hypothesis that SLCO4A1 is implicated in CRC progression. EMT, characterized by lower levels of epithelial markers and higher levels of mesenchymal markers, promotes cancer cell migration and invasion. Our results revealed that SLCO4A1 knockdown upregulated epithelial markers and downregulated mesenchymal markers in LoVo cells. Conversely, SLCO4A1 overexpression decreased E-cadherin levels and increased the levels of vimentin and N-cadherin in HT29 cells. These data suggested that SLCO4A1 controlled CRC cell invasion and migration by promoting EMT.

Accumulating evidence demonstrates that miRNAs are implicated in tumor initiation and progression. Bioinformatics analysis and luciferase reporter assays proved that SLCO4A1 was a target gene of miR-1224-5p. miR-1224-5p is downregulated in ovarian cancer [9], esophageal squamous cell carcinoma [21], pancreatic cancer [22], and rectal cancer [23]. starBase and RT-qPCR analyses showed that miR-1224-5p expression was low in CRC tissues and cell lines, consistent with a previous study [2]. miR-1224-5p mimic increased the mRNA and protein levels of SLCO4A1, whereas a miR-1224-5p inhibitor had the opposite effect, indicating that miR-1224-5p negatively regulated SLCO4A1. Rescue experiments were performed to investigate whether SLCO4A1 was regulated by miR-1224-5p. SLCO4A1 knockdown partially reversed the effect of the miR-1224-5p inhibitor on the phenotype of LoVo cells. Conversely, SLCO4A1 partially reversed the effect of miR-1224-5p mimic on the phenotype of HT29 cells. These findings suggested that SLCO4A1 was regulated by miR-1224-5p and that miR-1224-5p/SLCO4A1 could be considered a new strategy for CRC treatment.

This study demonstrated that SLCO4A1 was highly expressed in CRC, and SLCO4A1 silencing reversed the effects of miR-1224-5p on CRC cell proliferation, migration, invasion, and EMT. However, Buxhofer-Ausch et al. found that OATP4A1 expression levels were inversely correlated with tumor recurrence in early-stage CRC, indicating that OATP4A1 levels varied at different stages of cancer [24]. In addition, SLCO4A1 was identified as candidate transporter for erlotinib [25], implying that SLCO4A1 may be involved in drug resistance. In terms of these points, it deserves further study in the future. In conclusion, our data indicated that SLCO4A1 was a potential biomarker for the diagnosis and treatment of CRC.

Acknowledgments: This work was supported by the Zhejiang Province Traditional Chinese Medicine Science and Technology Project (2021ZB136, by Yingying Li).

References

[1] BRAY F, FERLAY J, SOERJOMATARAM I, SIEGEL RL, TORRE LA et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424. https://doi.org/10.3322/caac.21492

[2] LI J, PENG W, YANG P, CHEN R, GU Q et al. MicroRNA-1224-5p Inhibits Metastasis and Epithelial-Mesenchymal Transition in Colorectal Cancer by Targeting SP1-Mediated NF-kappaB Signaling Pathways. Front Oncol 2020; 10: 294. https://doi.org/10.3389/fonc.2020.00294

[3] DOSSA F, ACUNA SA, RICKLES AS, BERHO M, WEXNER SD et al. Association Between Adjuvant Chemotherapy and Overall Survival in Patients With Rectal Cancer and Pathological Complete Response After Neoadjuvant Chemotherapy and Resection. JAMA Oncol 2018; 4: 930–937. https://doi.org/10.1001/jamaoncol.2017.5597

[4] BAN MJ, JI SH, LEE CK, BAE SB, KIM HJ et al. Solute carrier organic anion transporter family member 4A1 (SLCO4A1) as a prognosis marker of colorectal cancer. J Cancer Res Clin Oncol 2017; 143: 1437–1447. https://doi.org/10.1007/s00432-017-2393-7

[5] WLCEK K, SVOBODA M, RIHA J, ZAKARIA S, OLSZEWSKI U et al. The analysis of organic anion transporting polypeptide (OATP) mRNA and protein patterns in primary and metastatic liver cancer. Cancer Biol Ther 2011; 11: 801–811. https://doi.org/10.4161/cbt.11.9.15176

[6] BUXHOFER-AUSCH V, NEMET O, SHEIKH M, ANDRIKOVICS H, REINER A et al. Two common polymorphic variants of OATP4A1 as potential risk factors for colorectal cancer. Oncol Lett 2020; 20: 252. https://doi.org/10.3892/ol.2020.12115

[7] BRENNER S, KLAMETH L, RIHA J, SCHOLM M, HAMILTON G et al. Specific expression of OATPs in primary small cell lung cancer (SCLC) cells as novel biomarkers for diagnosis and therapy. Cancer Lett 2015; 356: 517–524. https://doi.org/10.1016/j.canlet.2014.09.025

[8] WU K, XU T, SONG X, SHEN J, ZHENG S et al. LncRNA SLCO4A1-AS1 modulates colon cancer stem cell properties by binding to miR-150-3p and positively regulating SLCO4A1. Lab Invest 2021; 101: 908–920. https://doi.org/10.1038/s41374-021-00577-7

[9] WANG J, HU Y, YE C, LIU J. miR-1224-5p inhibits the proliferation and invasion of ovarian cancer via targeting SND1. Hum Cell 2020; 33: 780–789. https://doi.org/10.1007/s13577-020-00364-4

[10] LEE YS, DUTTA A. MicroRNAs in cancer. Annu Rev Pathol 2009; 4: 199–227. https://doi.org/10.1146/annurev.pathol.4.110807.092222

[11] ZHANG Z, LI J, HUANG Y, PENG W, QIAN W et al. Upregulated miR-1258 regulates cell cycle and inhibits cell proliferation by directly targeting E2F8 in CRC. Cell Prolif 2018; 51: e12505. https://doi.org/10.1111/cpr.12505

[12] XU F, YE ML, ZHANG YP, LI WJ, LI MT et al. MicroRNA-375-3p enhances chemosensitivity to 5-fluorouracil by targeting thymidylate synthase in colorectal cancer. Cancer Sci 2020; 111: 1528–1541. https://doi.org/10.1111/cas.14356

[13] CHEN X, XU X, PAN B, ZENG K, XU M et al. miR-150-5p suppresses tumor progression by targeting VEGFA in colorectal cancer. Aging (Albany NY) 2018; 10: 3421–3437. https://doi.org/10.18632/aging.101656

[14] LI JH, LIU S, ZHOU H, QU LH, YANG JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 2014; 42: D92–97. https://doi.org/10.1093/nar/gkt1248

[15] TANG Z, LI C, KANG B, GAO G, LI C et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98–W102. https://doi.org/10.1093/nar/gkx247

[16] SIEGEL RL, MILLER KD, GODING SAUER A, FEDEWA SA, BUTTERLY LF et al. Colorectal cancer statistics, 2020. CA Cancer J Clin 2020; 70: 145–164. https://doi.org/10.3322/caac.21601

[17] TAN B, PIWNICA-WORMS D, RATNER L. Multidrug resistance transporters and modulation. Curr Opin Oncol 2000; 12: 450–458. https://doi.org/10.1097/00001622-200009000-00011

[18] STIEGER B, HAGENBUCH B. Organic anion-transporting polypeptides. Curr Top Membr 2014; 73: 205–232. https://doi.org/10.1016/B978-0-12-800223-0.00005-0

[19] HAGENBUCH B, GUI C. Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. Xenobiotica 2008; 38: 778–801. https://doi.org/10.1080/00498250801986951

[20] THAKKAR N, LOCKHART AC, LEE W. Role of Organic Anion-Transporting Polypeptides (OATPs) in Cancer Therapy. AAPS J 2015; 17: 535–545. https://doi.org/10.1208/s12248-015-9740-x

[21] SHI ZZ, WANG WJ, CHEN YX, FAN ZW, XIE XF et al. The miR-1224-5p/TNS4/EGFR axis inhibits tumour progression in oesophageal squamous cell carcinoma. Cell Death Dis 2020; 11: 597. https://doi.org/10.1038/s41419-020-02801-6

[22] KONG L, LIU P, ZHENG M, WANG Z, GAO Y et al. The miR-1224-5p/ELF3 Axis Regulates Malignant Behaviors of Pancreatic Cancer via PI3K/AKT/Notch Signaling Pathways. Onco Targets Ther 2020; 13: 3449–3466. https://doi.org/10.2147/OTT.S248507

[23] SONG NS, PEI ZD, FU G. MiR-1224-5p acts as a tumor suppressor via inhibiting the malignancy of rectal cancer through targeting SLC29A3. IUBMB Life 2020; 72: 2204–2213. https://doi.org/10.1002/iub.2352

[24] BUXHOFER-AUSCH V, SHEIKH M, AUSCH C, ZOTTER S, BAUER H et al. Abundance of the Organic Anion-transporting Polypeptide OATP4A1 in Early-Stage Colorectal Cancer Patients: Association With Disease Relapse. Appl Immunohistochem Mol Morphol 2019; 27: 185–194. https://doi.org/10.1097/PAI.0000000000000557

[25] SHEN Y, YAN Z. Systematic prediction of drug resistance caused by transporter genes in cancer cells. Sci Rep 2021; 11: 7400. https://doi.org/10.1038/s41598-021-86921-9