Original Article

Lidocaine exerts anticancer activity in bladder cancer by targeting isoprenylcysteine carboxylmethyltransferase (*ICMT*)

Xiaodan Teng, Yang Liu, Liping Wang, Guonian Wang

Department of Anesthesiology, Harbin Medical University Cancer Hospital, Harbin, China

Contributions: (I) Conception and design: X Teng, Y Liu; (II) Administrative support: G Wang; (III) Provision of study materials or patients: Y Liu, L Wang; (IV) Collection and assembly of data: X Teng, L Wang; (V) Data analysis and interpretation: X Teng, Y Liu, G Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Guonian Wang. Harbin Medical University Cancer Hospital, No. 150 Haping Street, Nangang District, Harbin 150086, China. Email: t345254900@163.com.

Background: Bladder cancer is one of the most common malignant tumors among humans and has a high mortality. Clinically, lidocaine is the most commonly used local anesthetic, which can inhibit the proliferation of bladder cancer cells; however, its downstream specific molecular mechanisms are unclear.

Methods: The SwissTarget and TargetNet databases were used to analyze the target of lidocaine. The online public cancer transcriptome database UALCAN was used to analyze the up-regulated genes in The Cancer Genome Atlas Urothelial Bladder Carcinoma (TCGA-BLCA) data collection, and the intersection of the 2 was used to obtain the core target. The only target, isoprenylcysteine carboxylmethyltransferase (*ICMT*), was obtained by combining the correlation between the target and the clinical information of bladder cancer and the Kaplan-Meier (K-M) survival curve. Then, UMUC3 and T24 cells were selected as research vectors *in vitro*. Cell proliferation, cell cycle, and apoptosis were detected by cell counting kit-8, colony formation, flow cytometry, and western blotting.

Results: Network pharmacology analysis showed that *ICMT* might be one of the targets of lidocaine, and the expression level of *ICMT* was closely related to the clinical phenotype of bladder cancer. Lidocaine treatment (4 and 8 mM) significantly inhibited the proliferation of UMUC3 and T24 cells, promoted apoptosis, and significantly inhibited the mass and volume of xenograft tumors. *In vitro* experiments showed that *ICMT* promoted the proliferation of UMUC3 and T24 cells. Lidocaine inhibited the expression of *ICMT* in UMUC3 and T24 cells in a concentration and time-dependent manner, and inhibited cell proliferation by down-regulating *ICMT* expression.

Conclusions: Lidocaine exerts anti-tumor effect by down-regulating the expression of *ICMT* in bladder cancer.

Keywords: Bladder cancer; lidocaine; isoprenylcysteine carboxylmethyltransferase (ICMT); proliferation; apoptosis

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Introduction

Bladder cancer is one of the most common malignant tumors of the urinary system and the 11th most frequently diagnosed cancer in the world (1). Although significant advances have been made in surgical techniques and adjuvant therapy, the prognosis of bladder cancer remains poor. Therefore, understanding the molecular mechanism of bladder cancer is helpful to the exploration of effective diagnostic and prognostic markers of bladder cancer.

Lidocaine is a local anesthetic, which is widely clinically used in local anesthesia and analgesia. Recently, it has been found that subcutaneous infusion of lidocaine can be safely

used for cancer pain management (2). In addition, lidocaine also has good anti-tumor therapeutic potential. Lidocaine reduces the survival rate and colony formation ability of HepG2 cells by up-regulating the expression of CPEB3 (3). In human thyroid carcinoma cells, lidocaine induces apoptosis by regulating mitogen activated protein kinase (MAPK) pathway (4). Lidocaine can inhibit the proliferation of bladder cancer cells alone and enhance the anticancer effect of other chemotherapeutic drugs (5). However, the downstream molecular mechanism of lidocaine in regulating the proliferation of bladder cancer cells is unclear.

The final step of isopreanoidization of translated proteins catalyzed by isoprenylcysteine carboxylmethyltransferase (*ICMT*) is crucial to the stability and normal function of many carcinogenic proteins. Recent studies have shown that the expression of *ICMT* is up-regulated in ovarian cancer and cervical cancer, and that it promotes a variety of malignant biological behaviors of tumor cells, such as growth, migration, and survival (6-8). Consistently, *ICMT* inhibition has exhibited anticancer activity in cancers including pancreatic cancer, leukemia, and cervical cancer (9-11).

In this study, we obtained *ICMT* as one of the targets of lidocaine in bladder cancer patients through network pharmacology analysis. The expression of *ICMT* in tissue samples of bladder cancer patients analyzed from The Cancer Genome Atlas (TCGA) database was significantly increased, and the high expression of *ICMT* was related to poor tumor grade, poor pathological T stage, and high recurrence rate. *In vitro*, *ICMT* promotes the proliferation of bladder cancer cells, whereas lidocaine inhibits cell survival and growth by downregulating *ICMT*. In this study, we investigated the mechanism of lidocaine on bladder cancer cell proliferation. This study has laid the foundation for lidocaine treatment of bladder cancer. We present the following article in accordance with the ARRIVE reporting checklist (available at https://dx.doi.org/10.21037/tau-21-893).

Methods

Computational target fishing using TargetNet and SwissTarget

The SDF file of lidocaine (Pubchem CID: 3676) was downloaded from the Pubchem database and entered into TargetNet (https://targetnet.scbdd.com/) and SwissTarget (https://www.swisstargetprediction.ch/) servers. The default values were set for all parameter settings, and the protein targets identified by the 2 servers were allowed to overlap. Therefore, they can be considered as preselected targets for further research.

UALCAN database

The online public cancer transcriptome database UALCAN (ualcan.path.uab.edu/) was used to analyze the up-regulated genes in The Cancer Genome Atlas Urothelial Bladder Carcinoma (TCGA-BLCA). According to the clinicopathological parameters, such as tumor T stage and tumor grade, the gene expression profiles in bladder cancer samples were analyzed, and the target genes were screened. Subsequently, Kaplan-Meier (K-M) survival analysis was performed on the target genes to explore the value and significance of a single gene in the prognosis prediction of bladder cancer. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Cell culture and transfection

Human bladder cancer cell lines (UMUC3 and T24) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured at 37 ℃ in 5% CO_2 in Roswell Park Memorial Institute (RPMI)-1640 culture medium (Gibco, Gaithersburg, MD, USA) containing 10% fetal bovine serum (FBS; Gibco), and subcultured at 80–90% cell density. According to the standard procedure, *ICMT* overexpression plasmid (Addgene, Watertown, MA, USA; Plasmid # 29272) was transfected into cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Then, *ICMT* short hairpin RNA (shRNA) expressing plasmids were constructed in lentiviral vector PLL3.7. by (GenePharma, Shanghai, China). The *ICMT* target sequence was: (I) 5'-CCCTGTCATTGTTCCACTATT-3', (II) 5'-CTTGGTTTCGGCATCCTTCTT-3'. Cells were harvested for *ICMT* protein expression level analysis at 72 h post transfection.

Cell proliferation test

Cell counting Kit-8 (CCK-8; Beyotime, Shanghai, China) was used to explore cell proliferation. About 1×10^4 cells or transfected cells were seeded into triplicate wells of 96-well plates with 100 μL medium per well. After 24 h of culture, cells were treated with different doses of lidocaine (Sigma-

Aldrich, St. Louis, MO, USA) at different time points. The cells were then incubated with 10 μL CCK-8 solution for 2 h, and the absorbance was measured at 450 nm.

Colony formation assay

After 48 h of culture with untreated, treated with lidocaine, or transfected cells, the cells were seeded into each well of the 6-well culture plate at a density of 500 cells/well. After 14 days of culture in drug-free culture, the cells were fixed with paraformaldehyde for 15 min, and stained with crystal violet for 10 min to observe the colonies. Only ≥50 cells were counted and compared as positive colonies.

Flow cytometry detection of cell cycle distribution

For cell cycle analysis, the specified treated UMUC3 and T24 cells were harvested and fixed overnight in 70% ethanol at 4 ℃. The cell lines were treated with 0.5 mg/mL RNase A (Keygen Biotech, Nanjing, China) and stained with 1 mg/mL propidium iodide (PI; Becton, Dickinson and Co. Biosciences (BD), San Diego, CA, USA) for 0.5 h at 37 ℃. We then used FACSCalibur flow cytometry (BD, San Jose, CA, USA) was used to determine the distribution of cell cycle stages. The percentages of G0/G1, S, and G2/M cells were calculated.

Flow cytometry detection of apoptosis

For apoptosis analysis, UMUC3 and T24 cells were harvested and washed twice with cold phosphate-buffered saline (PBS) and suspended in binding buffer containing V-FITC and PI for 15 min at room temperature. The FACSCalibur flow cytometry was used to analyze apoptosis.

Western blot analysis

The cells to be tested were lysed in radioimmunoprecipitation assay (RIPA) buffer (Beyotime) and centrifuged at 12,000 rpm for 15 min at 4 ℃. The supernatant was collected and the total protein content was determined by bicinchoninic acid (BCA) Protein Assay Kit (Beyotime). The same protein (40 μg/lane) was separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to polyvinylidene fluoride (PVDF) membrane (Millipore, Danvers, MA, USA). The membrane was sealed with 5% skimmed milk and incubated with primary antibodies against Ki67 (1:3,000; ab92742, Abcam, Cambridge, MA, USA), CDK2 (1:1,000; ab32147, Abcam), CCNB1 (1:10,000; ab32053, Abcam), BAX (1:1,000; ab32503, Abcam), BCL2 (1:500; ab32124, Abcam), and β-actin (1:2,000; ab6276, Abcam) overnight at 4 ℃. The next day, after washing with PBS/Tween (PBST) for 3×5 min, the membrane was incubated with secondary antibody at 37 ℃ for 2 h. After washing, Tanon-5200 image analyzer (Tanon Science and Technology, Shanghai, China) was used to quantify protein bands.

Lactate dehydrogenase (LDH) cytotoxicity test

LDH assay was performed using D-Plus TM LDH cytotoxicity assay kit (Dongin Biotech, Seoul, Korea). In short, 2.5×10^4 cells per well were seeded in 96-well plates. After incubation for 24 h, the specified dose of lidocaine was added to each hole, and the final volume was 100 μL. After 48 h, the solution was centrifuged at 600 ×g for 10 min to remove floating cells from the supernatant. The control cells were lysed by adding 10 μL lysis buffer before centrifugation. Each supernatant (10 μL) was transferred to a new hole in the 96-well plate. Finally, 100 μL LDH reaction mixture (the ratio of WST substrate to LDH determination buffer was 1:50) was added, and the samples were incubated at 25 ℃ for 30 min. The absorbance at 450 nm was measured by a microplate reader. We conducted 3 independent experiments in duplicate at different time points.

Nude mice xenograft model

A total of 16 male BALB/c-nu/nu mice, aged 4–6 weeks and weighing 18–22 g, were purchased from Vital River Laboratories (Beijing, China). They were raised under specific pathogen free (SPF) conditions with a 12-h light/dark cycle in a temperature-controlled facility (22 ℃) with access to chow and water ad libitum. Mice were randomly divided into 2 groups: control group and lidocaine (8 mice in each group). Mice in the lidocaine group were injected with 5×10^6 T24 cells into the right abdominal cavity, and lidocaine (100 mg/kg) was intravenously injected 3 times every week (12). The mice in the control group were injected with T24 cells and an equal volume of normal saline 3 times in the right abdominal cavity. After 14 days, the mice were sacrificed by cervical dislocation. Calculation of tumor volume and weight was performed as described above (13). Experiments were performed under a project license (No. 2020067) granted by ethics committee of

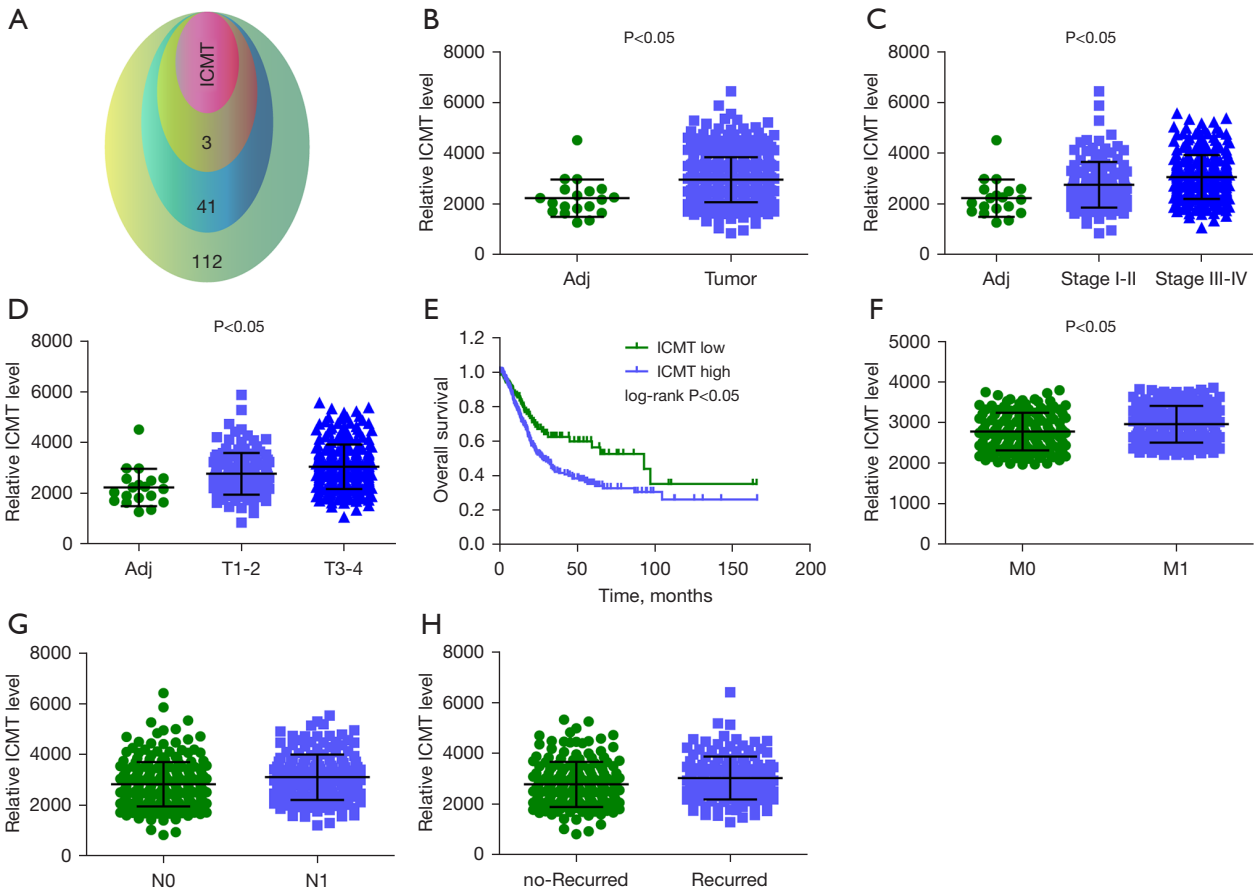

Figure 1 Network pharmacology analysis of lidocaine targets in bladder cancer. (A) The prediction schematic diagram of the core target of lidocaine; (B) the expression of *ICMT* in TCGA-BLCA; (C) the expression of ICMT and the tumor grade of BLCA; (D) the expression of ICMT and the T stage of BLCA tumor; (E) K-M curve was used to analyze the relationship between the expression of ICMT and the survival of BLCA. The expression level of ICMT was correlated with distal metastasis (F), lymph node metastasis (G) and recurrence (H) of bladder cancer. P<0.05 was considered statistically significant. TCGA-BLCA, The Cancer Genome Atlas Urothelial Bladder Carcinoma; K-M, Kaplan-Meier.

Harbin Medical University Cancer Hospital, in compliance with Animal Management Rule of China (Ministry of Health, China, document No. 552001). A protocol was prepared before the study without registration.

Statistical analysis

Data were expressed as mean ± SD for at least 3 independent experiments. The software SPSS 19.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The statistical evaluation of the data was carried out by using unpaired Student's *t*-test and analysis of variance (ANOVA) by a post-hoc test. A P value <0.05 was considered t statistically significant and graphs were created with GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA).

Results

Network pharmacology predicts the target of lidocaine and the clinical significance of ICMT in bladder cancer patients

We used SwissTarget (pro >0) and TargetNet (pro >0) to predict the target of lidocaine. The 2 were combined to obtain 157 targets of lidocaine (*Figure 1A*). The UALCAN online database was used to analyze the up-regulated genes in TCGA-BLCA, and the results showed that 45 genes, including *ICMT*, were up-regulated (*Figure 1B*).

The TCGA-BLCA data matrix and clinical information were downloaded to analyze the relationship between the expression levels of these 44 genes and the tumor grade and T staging of BLCA. The results showed that only *ICMT*, *DHODH*, *DRD4*, and *FAP* increased with the progression of tumor grade and T staging. The expression trend of *ICMT* is shown in *Figure 1C,1D*. In addition, we further analyzed the relationship between these 4 genes and BLCA survival. The K-M curve showed that only *ICMT* was associated with BCLA survival (*Figure 1E*). Therefore, this study selected *ICMT* as the research object. Subsequently, we found that the higher the expression of *ICMT*, the higher the incidence of distant metastasis, lymph node metastasis, and recurrence rate of bladder cancer (*Figure 1F-1H*).

Inhibition of proliferation and cycle of bladder cancer cells by lidocaine in vitro

The chemosensitivity of UMUC3 and T24 cells to lidocaine was determined by CCK-8 assay. The results showed that lidocaine inhibited the survival of UMUC3 and T24 cells, and the inhibition rate increased with the increase of drug treatment time and concentration. The UMUC3 and T24 cells were treated with 4 and 8 mM lidocaine for 48 h, respectively, and the cell inhibition rate reached about 50% (*Figure 2A,2B*). Therefore, UMUC3 and T24 cells were treated with lidocaine at the concentrations of 4 and 8 mM for 48 h, respectively. As shown in *Figure 2C,2D*, compared with the control group, lidocaine significantly reduced the optical density (OD) value. In addition, we evaluated the colony formation ability of the treated cells. As shown in *Figure 2E*, lidocaine at 4 and 8 mM for 48 h significantly reduced the colony formation ability of UMUC3 and T24 cells, indicating that lidocaine inhibited the proliferation of bladder cancer cells. Next, in order to investigate whether lidocaine mediates the inhibitory effect of bladder cancer cell growth through cell cycle arrest mechanism, we used flow cytometry (PI staining) to analyze the cell cycle distribution after 48 h of treatment with the specified concentration of lidocaine. As shown in *Figure 2F,2G*, compared with the control group, lidocaine treatment significantly increased the proportion of cells in G0/G1 phase and decreased the proportion of cells in S phase, suggesting that lidocaine inhibited the growth of bladder cancer cells, partly due to the induction of cell cycle arrest in G1/S phase. Secondly, lidocaine significantly inhibited the protein expression of Ki67, CDK2, and CCNB1 in UMUC3 and T24 cells (*Figure 2H*).

Lidocaine promotes apoptosis of bladder cancer cells in vitro

The LDH cytotoxicity test is a colorimetric method, which provides a simple and reliable method for the determination of cytotoxicity. In UMUC3 and T24 cells, the LDH cytotoxicity of cells treated with the specified concentration of lidocaine was significantly higher than that of the control group (*Figure 3A*). Then, flow cytometry was used to detect the apoptosis of UMUC3 and T24 cells after lidocaine treatment. Lidocaine significantly increased the proportion of apoptotic cells in UMUC3 and T24 cells (*Figure 3B*). In order to further study the mechanism of this phenomenon, the expression level of apoptosis-related proteins was measured by western blotting. After lidocaine treatment, BAX levels in UMUC3 and T24 cells increased, while BCL2 levels decreased (*Figure 3C*), indicating that lidocaine may induce apoptosis by activating endogenous apoptotic pathways.

Lidocaine inhibits bladder cancer cell proliferation in vivo

To evaluate the antitumor effect of lidocaine *in vivo*, a T24 cell subcutaneous transplantation model was established. The body weight and tumor volume of mice were positively correlated with tumor severity. As shown in *Figure 4*, the tumor volume (*Figure 4B,4C*) and tumor weight (*Figure 4D*) of the control animals transplanted with T24 cancer cells were increased. Compared with the control group, the tumor volume (*Figure 4B,4C*) and tumor weight (*Figure 4D*) in the lidocaine group were significantly decreased.

ICMT promotes bladder cancer cell proliferation in vitro

In order to understand the effect of *ICMT* on the proliferation of bladder cancer cells, we used the methods of functional loss and functional acquisition to detect the proliferation. As shown in *Figure 5*, we found that *ICMT* overexpression significantly increased the OD values of UMUC3 and T24 cells (*Figure 5B,5C*), promoted the cell colony formation ability (*Figure 5D*), and up-regulated the Ki67 protein level (*Figure 5E*), suggesting that overexpression of *ICMT* gene significantly promoted cell survival and proliferation. Contrary to the promotion of overexpression of *ICMT*, knockdown of ICMT by shRNA significantly decreased the OD value of UMUC3 and T24 cells (*Figure 5G,5H*), inhibited the colony formation ability

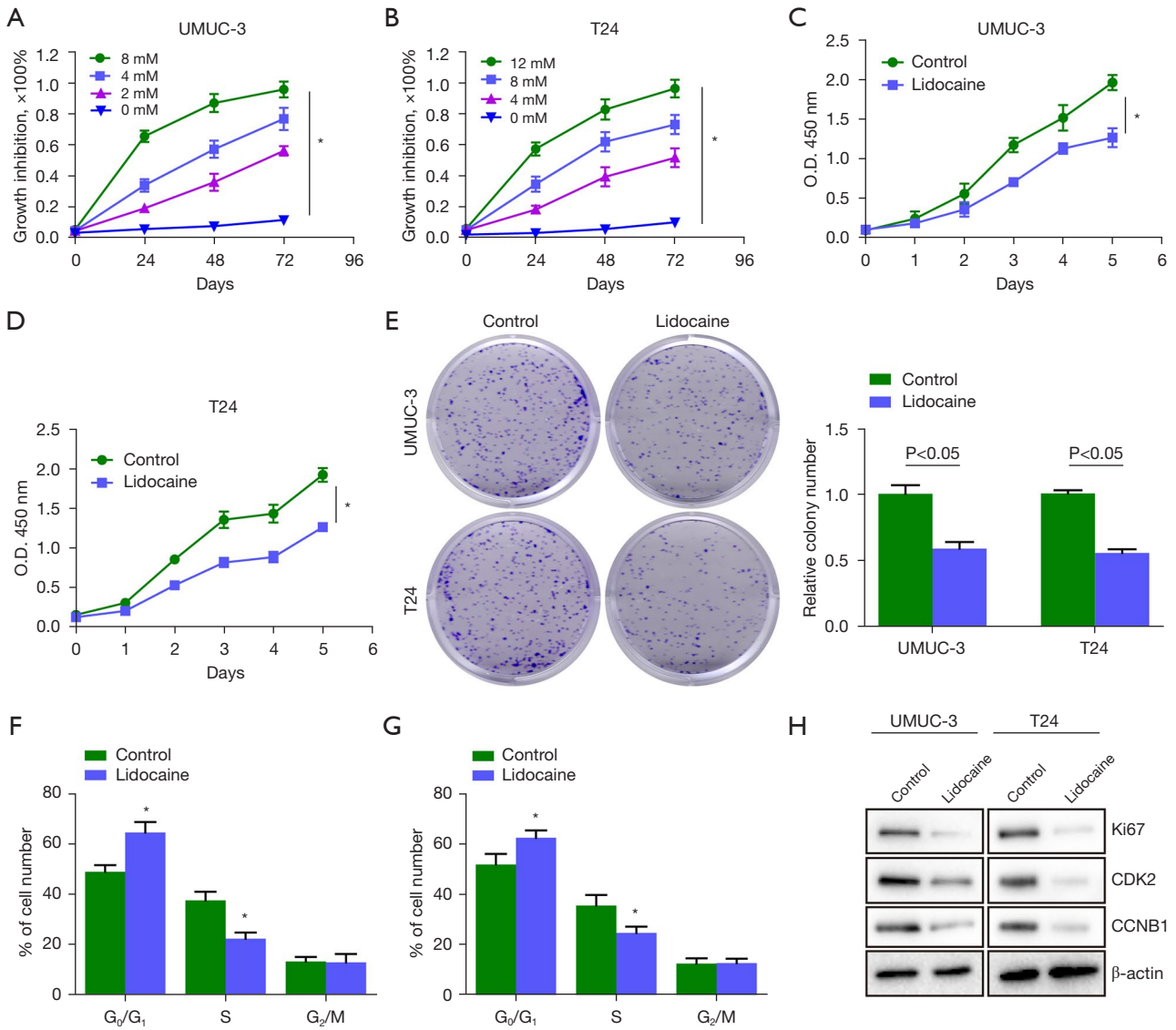

Figure 2 Effects of lidocaine on the proliferation and cycle of bladder cancer cells *in vitro*. (A,B) CCK-8 was used to detect the survival rates of UMUC3 and T24 cells after 24, 48, and 72 h treatment with different concentrations of lidocaine; (C,D) CCK-8 was used to detect the OD values of UMUC3 and T24 cells treated with 4 and 8 mM lidocaine at different time points; (E) a colony formation assay (crystal violet staining) was performed to determine the clonogenic capacity of UMUC3 and T24 cells; (F,G) quantification of G0/G1, S, and G2/M phase cells in lidocaine-treated UMUC3 and T24 cells at the indicated concentrations (PI staining); (H) expression levels of ki67, CDK2, and CCNB1 were examined by western blotting in UMUC3 and T24 cells treated with lidocaine for 48 h at different concentrations. β-actin was used as the loading control. Data are presented as the mean ± SD. *, P<0.05 *vs.* control. Results are representative of at least 3 independent experiments. CCK-8, cell counting kit-8; OD, optical density; PI, propidium iodide.

of cells (*Figure 5I*), and down-regulated the protein level of Ki67 (*Figure 5J*). In summary, these results clearly indicate that *ICMT* promotes the proliferation of bladder cancer cells.

Lidocaine inhibits bladder cancer cell proliferation through ICMT

Next, in order to further reveal the potential mechanism of lidocaine affecting UMUC3 and T24 cells, the protein

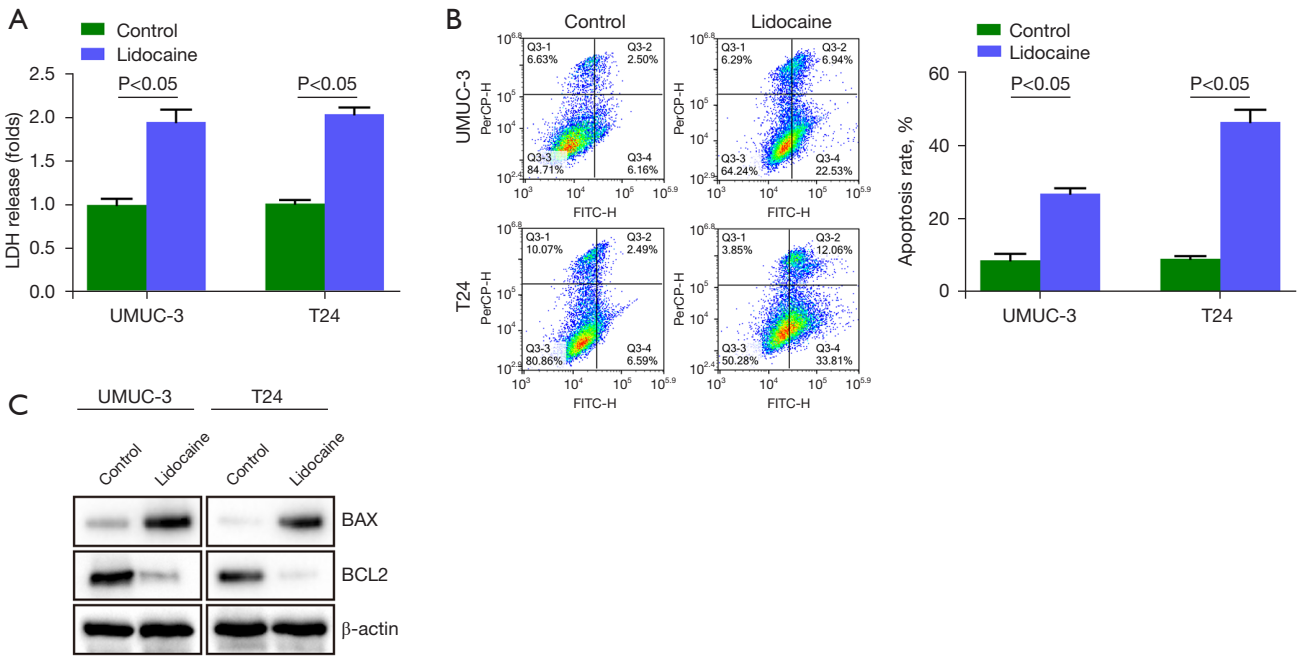

Figure 3 Effect of lidocaine on apoptosis of bladder cancer cells *in vitro*. (A) The cytotoxicity of lidocaine was evaluated by measuring the release of LDH. (B) The apoptosis induced by lidocaine was detected by annexin v-FITC and PI double staining. (C) Western blot was used to detect the expression levels of BAX and BCL2. β-actin was used as the loading control. Data are presented as the mean ± SD. *, P<0.05 *vs.* control. Results are representative of at least 3 independent experiments. LDH, lactate dehydrogenase; PI, propidium iodide.

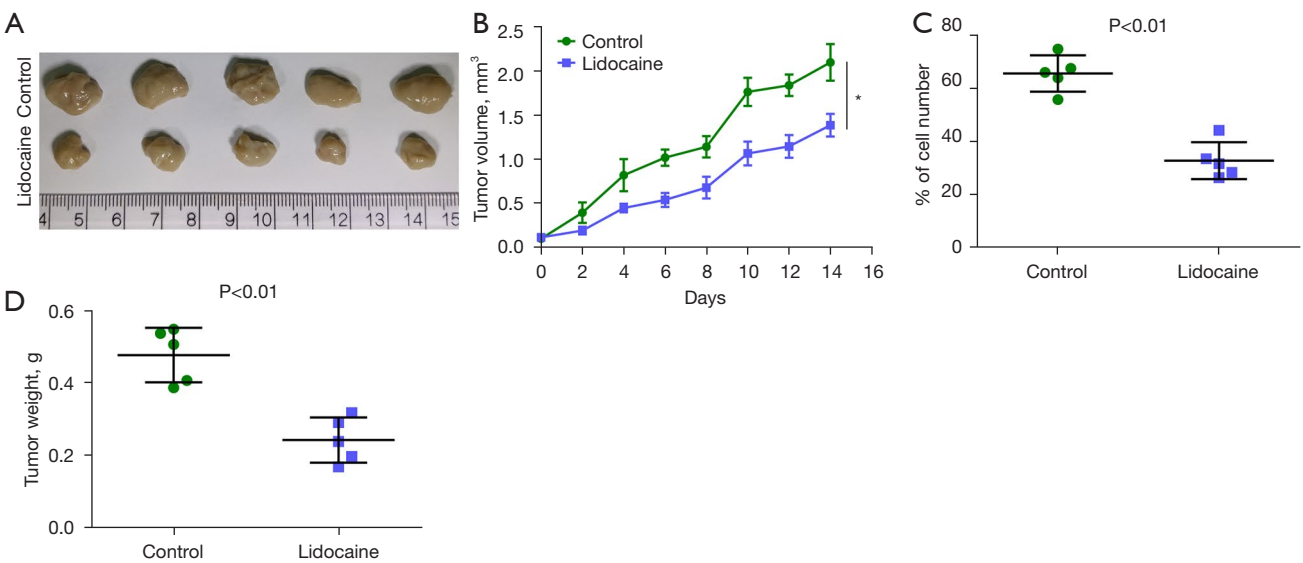

Figure 4 Antitumor effect of lidocaine on T24 tumor bearing nude mice. T24 cells were injected into right abdominal cavity of 4–6-week-old male nude mice. After injection of T24 cells, the nude mice were treated with normal saline and lidocaine (100 mg/kg) for 14 days, 3 times a week. (A) Tumor schematic diagram of nude mice 14 days after administration; (B) changes of tumor volume in nude mice within 14 days; (C) tumor volume on day 14; (D) tumor weight on day 14. Data are presented as the mean ± SD, n=5. *, P<0.05 *vs.* control.

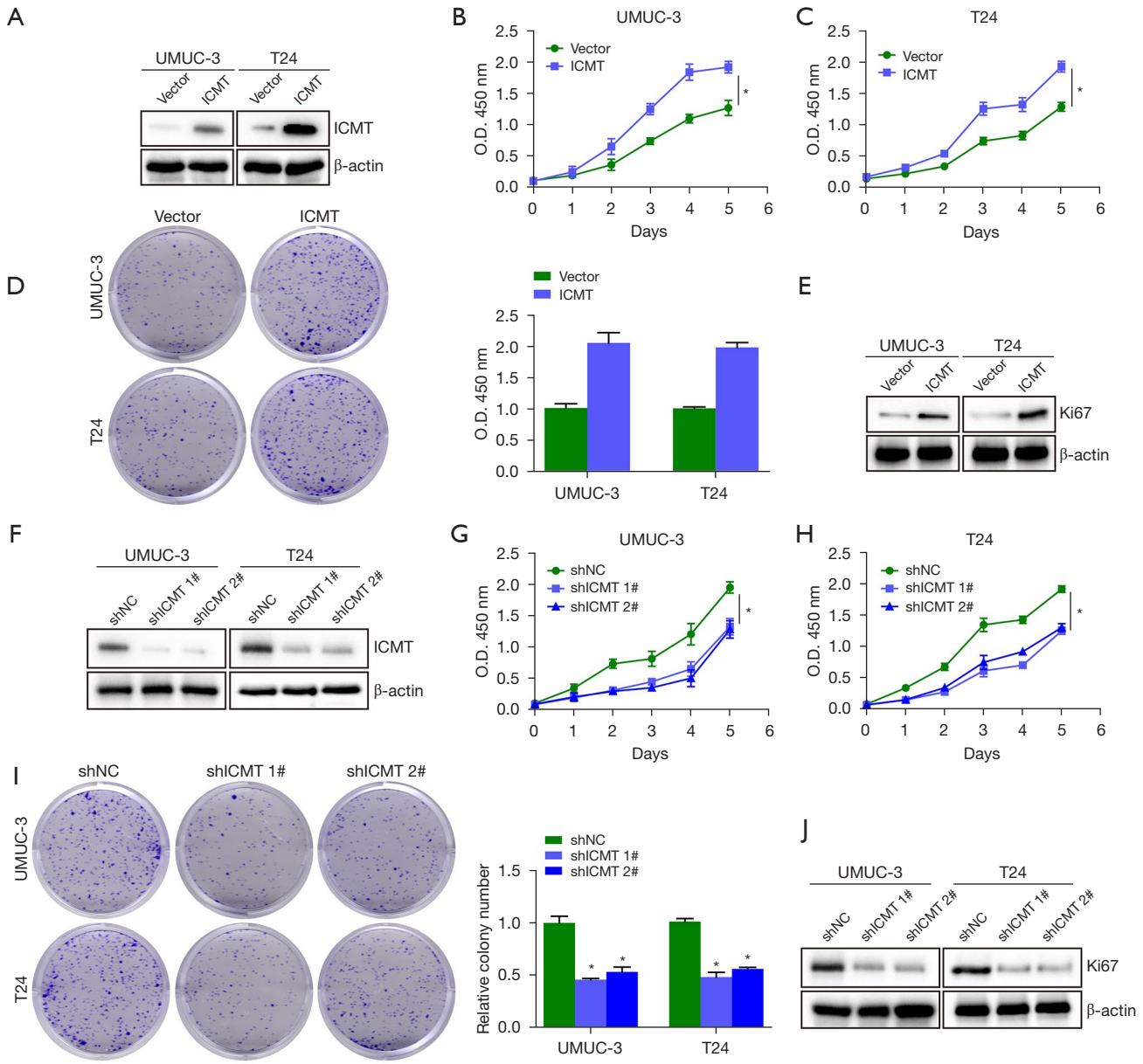

Figure 5 Effect of ICMT expression on the proliferation of bladder cancer cells. UMUC3 and T24 cells were transfected with ICMT overexpression or knockdown plasmids. Western blotting was used to analyze the protein expression levels of ICMT (A,F) and Ki67 (E,J). CCK-8 was used to detect the OD values of UMUC3 (B,G) and T24 cells (C,H). Colony formation assay (crystal violet staining) was used to determine the colony formation ability (D,I) of UMUC3 and T24 cells. Data are presented as the mean ± SD. *, P<0.05 *vs.* control. Results are representative of at least 3 independent experiments. CCK-8, cell counting kit-8; OD, optical density.

expression of ICMT was detected by western blot analysis. As shown in *Figure 6A*, in response to lidocaine treatment, the expression levels of ICMT in UMUC3 and T24 cells decreased in a concentration-dependent manner. Then, the expression changes of ICMT in UMUC3 and T24 cells treated with 4 and 8 mM lidocaine for 12, 24, and 48 h were detected. It was observed that lidocaine down-regulated the expression level of ICMT in a time-dependent manner (*Figure 6B*), suggesting that the expression of ICMT was regulated by lidocaine. We further treated cells with

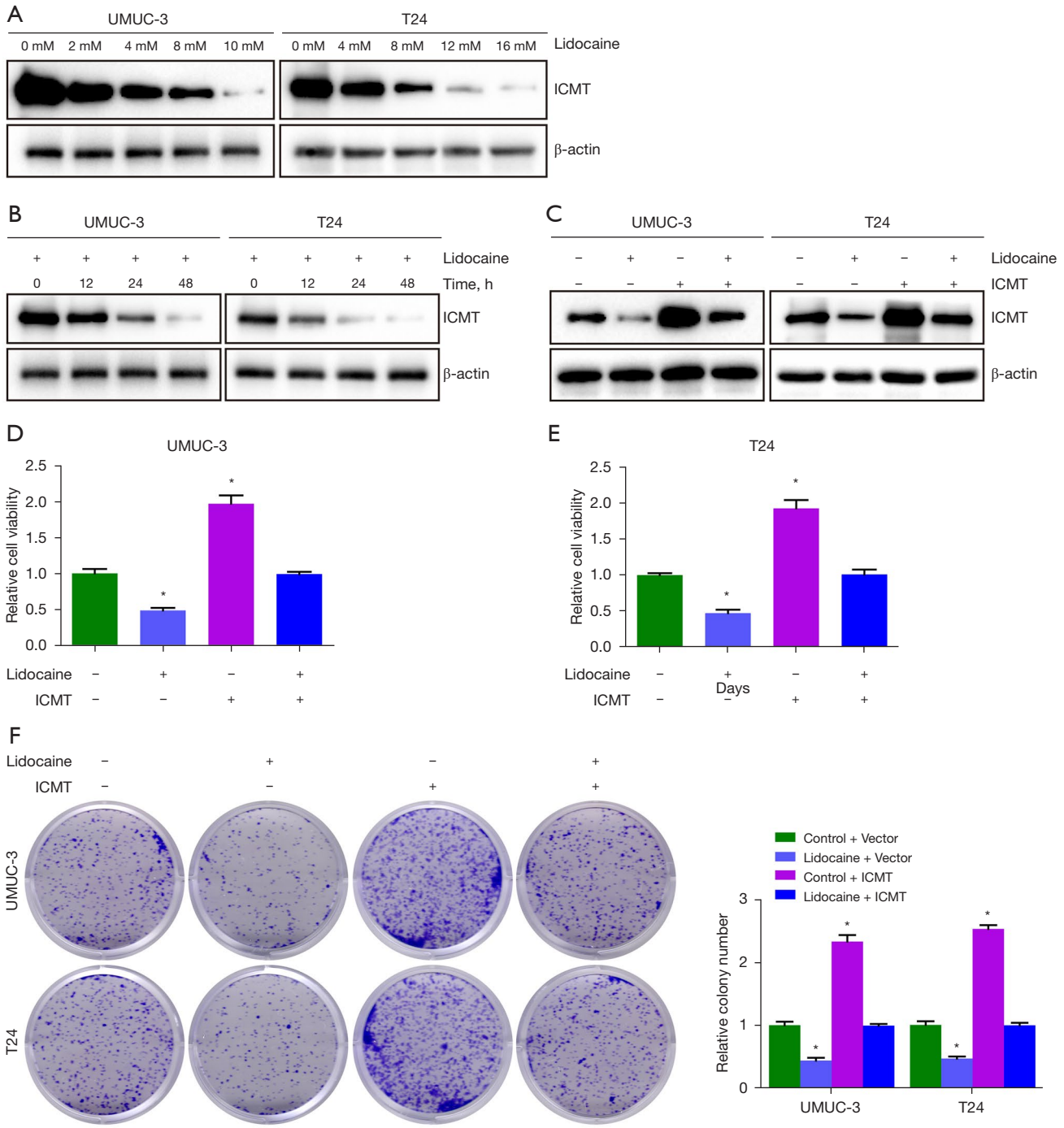

Figure 6 Lidocaine inhibits bladder cancer cell proliferation through ICMT. (A) After UMUC3 and T24 cells were treated with different concentrations of lidocaine, the protein level of *ICMT* was detected by western blotting; (B) after UMUC3 and T24 cells were treated with 4 and 8 mM lidocaine for different times, ICMT protein levels were detected by western blotting. After the cells were treated with lidocaine and *ICMT* overexpression, respectively or simultaneously; (C) Western blot was used to detect the protein level of *ICMT*; (D,E) the survival of UMUC3 and T24 cells was measured using CCK-8 analysis; and (F) colony formation experiment (crystal violet staining) was used to determine the colony formation ability of UMUC3 and T24 cells. *, P<0.05 *vs.* Control + Vector group.

lidocaine and ICMT overexpression, respectively or simultaneously, and the ICMT protein was successfully overexpressed (*Figure 6C*). Then we further measured cell survival and colony formation. The results showed that ICMT overexpression reversed the inhibition of lidocaine on cell survival and colony formation (*Figure 6D-6F*). In summary, these results confirmed that lidocaine inhibited cell proliferation by inhibiting ICMT expression.

Discussion

Bladder cancer is one of the most fatal malignant tumors in humans, with high incidence, high recurrence rate, and high mortality. Over the past 2 decades, local or metastatic bladder cancer has a poor prognosis and a low 5-year survival rate due to uncontrolled proliferation, apoptosis, and metastasis of tumor cells (14). Studies have shown that local anesthetics may be beneficial to the treatment of cancer. Lidocaine is the most commonly used local anesthetic in clinic, which can inhibit the proliferation, invasion, and migration of tumor cells and induce apoptosis of tumor cells (4,15). Recent studies have found that lidocaine can inhibit the proliferation of human bladder cancer cells, but there have been few studies on its specific downstream molecular mechanism (5). In this study, CCK-8 and cell colony analysis showed that the IC_{50} values (48 h) in UMUC3 and T24 cells were 3.92 and 7.84 mM, respectively. After 48 h treatment with 4 and 8 mM lidocaine, the survival rate and colony formation ability of tumor cells decreased significantly in a dose- and time-dependent manner. The results *in vivo* showed that lidocaine significantly inhibited the tumor growth of tumor mice, confirming the anti-tumor effect of lidocaine in bladder cancer. In addition, LDH cytotoxicity test was used to evaluate the cytotoxicity of lidocaine in 2 cell lines. The results showed that lidocaine significantly increased LDH levels in UMUC3 and T24 cells. In order to clarify the mechanism of anti-proliferation effect of lidocaine on bladder cancer cells, we studied the effect of lidocaine treatment on cell cycle progression of UMUC3 and T24. The results of flow cytometry showed that lidocaine caused cell cycle arrest in G1. Therefore, it can be inferred that the inhibition of proliferation of bladder cancer cells by lidocaine is caused by cell cycle arrest. This is consistent with the results reported by Chang *et al*. (15) that lidocaine reduces the viability of thyroid cancer cells through G1 arrest. Western blot analysis showed that lidocaine treatment significantly down-regulated the expression of ki67, CDK2, and CCNB1 proteins, indicating that lidocaine-induced cell cycle arrest may be achieved by regulating cyclin family and CDK family proteins.

Network pharmacology and bioinformatics analysis of TCGA patient samples provide a new perspective for the detection and treatment of bladder cancer. Previous studies have shown that *ICMT* is involved in the growth, drug resistance, and progression of many cancers (16-18). In our study, we found that *ICMT* is one of many targets of lidocaine, and the expression of *ICMT* is up-regulated in TCGA-BLCA patients, suggesting that the abnormal expression of *ICMT* is related to tumorigenesis. We further found that *ICMT* was up-regulated in the late stage of bladder cancer and related to adverse pathological T staging. High expression of *ICMT* is associated with poor survival and high recurrence rate. In addition, the effect of *ICMT* expression on the proliferation of bladder cancer cells was verified by *in vitro* transfection of *ICMT* overexpression and knock-down plasmid. This study found that overexpression of the *ICMT* gene can significantly promote the proliferation of UMUC3 and T24 cells. On the contrary, knockdown of the *ICMT* gene inhibits cell proliferation, which is consistent with the results of Xu *et al*. (18). These results suggest that *ICMT* may be a potential biomarker for the diagnosis and treatment of bladder cancer.

The direct effects of lidocaine on cancer cells include inhibiting proliferation, inducing apoptosis, and inhibiting metastasis efficiency (15,19,20). In particular, proliferation and apoptosis are 2 important signs of cancer cells, and inhibiting cell proliferation and inducing apoptosis are 2 promising strategies for anti-tumor development (21). The BCL2 family plays an important role in apoptosis, including anti-apoptotic proteins (BH1–4 domain) and pro-apoptotic proteins (BH1–3 and BH3 domain) (22). Specifically, the BCL2 subfamily is associated with inhibition of apoptosis, while the Bax subfamily promotes apoptosis (23). In this study, lidocaine treatment induced the apoptosis of UMUC3 and T24 cells. The mechanism may be through up-regulating the expression of pro-apoptotic protein Bax and down-regulating the expression of anti-apoptotic protein Bcl-2, thereby activating the endogenous apoptotic pathway to trigger apoptosis. This is consistent with the results of lidocaine inducing HepG2 cell apoptosis by increasing the Bax/Bcl-2 ratio (4).

It is further speculated that lidocaine may inhibit the proliferation of bladder cancer cells by targeting *ICMT*.

Our rescue experiments showed that the recovery of *ICMT* attenuated the inhibitory effect of lidocaine on the survival and growth of UMUC3 and T24 cells. The novelty of this study includes network pharmacology analysis of *ICMT* as a target gene of lidocaine and its clinical significance, application of 2 bladder cancer cell lines and rescue experiments. In summary, *ICMT* is a medium for lidocaine to inhibit the proliferation of bladder cancer cells. And We can screen and identify more key biomarkers of bladder cancer through TCGA and GEO data and a series of bioinformatics analysis.

Conclusions

Taken together, our results suggest that lidocaine affects the proliferation of bladder cancer cells by regulating cell cycle and Bax/Bcl-2 ratio. Lidocaine can down-regulate *ICMT* to inhibit the proliferation of bladder cancer cells.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tau-21-893). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Experiments were performed under a project license (No. 2020067) granted by ethics committee of Harbin Medical University Cancer Hospital, in compliance with Animal Management Rule of China (Ministry of Health, China, document No. 552001).

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