Therapeutic Effect of Sodium Glucose Co-Transporter 2 Inhibitor Dapagliflozin on Renal Cell Carcinoma

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Source of support: This work was supported by the Science and Technology Foundation of Zhuhai (No. 2015A0005)

Background: Patients with type 2 diabetes mellitus (T2DM) have a high incidence of renal cell carcinoma (RCC) and high sodium glucose co-transporters 2 (SGLT2) expressions. The purpose of this study was to evaluate the anticancer activity of dapagliflozin as an SGLT2 inhibitor on RCC cell lines *in vitro* and *in vivo*.

Material/Methods: qRT-PCR and Western blot were used to detect SGLT2 expression on different human renal cells. Then, flow cytometry and immunofluorescence were used to investigate the effects of dapagliflozin on cell cycle, apoptosis, and SGLT2 expression of CaKi-1 cells. Finally, a xenograft model and immunohistochemical staining were used to investigate the function of dapagliflozin in nude mice.

Results: We proved that SGLT2 is highly expressed in RCC cell lines. We found that dapagliflozin exerts a higher cytotoxic effect on human RCC than on normal human renal cells, regulates the cell cycle and apoptosis, and reduces the glucose uptake and SGLT2 expression of CaKi-1 cells. Moreover, dapagliflozin inhibits tumor growth and reduces SGLT2 expression *in vivo*.

Conclusions: Our results indicate that dapagliflozin has high efficiency and low toxicity and could be a new therapeutic target for RCC.

MeSH Keywords: **Carcinoma, Renal Cell • Diabetes Mellitus, Type 2 • Sodium-Glucose Transporter 2**

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/902530

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Background

Along with the improvement of living standards and change of lifestyle, the number of people with diabetes mellitus worldwide has increased sharply. In China, the prevalence of diabetes in adults was only 2.7% according to the 2002 National Nutrition Survey [1], but in the 2010 Chronic Disease Risk Factor Surveillance, the prevalence had increased to 9.7% according to the same criteria [2]. Patients with type 2 diabetes mellitus (T2DM) account for 90–95% of newly diagnosed cases; these people are at increased risk of developing serious health complications, such as diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, cardiovascular diseases, and diabetic neuropathic osteoarthropathy [3–5]. In addition, T2DM conveys an increased risk of the occurrence and development of malignant tumors, such as kidney cancer, colorectal cancer, bladder cancer, pancreatic cancer, and breast cancer [2]. Therefore, T2DM and its complications have a significantly economic impact on individuals, families, health systems, and countries, and diabetes complications greatly affect clinical practice.

T2DM is refractory to medical therapy and has a complex etiology. Although patients have many options available regarding anti-diabetic agents; it' i hard to obtain satisfactory blood glucose control. Sodium glucose co-transporters (SGLT) are a family of glucose transporters found in the intestinal mucosa of the small intestine and the proximal tubule of the nephrons. Human SGLT is mainly composed of SGLT1 and SGLT2. Due to the specific distribution and the high efficiency of SGLT2, an SGLT2 inhibitor is a potential new strategy for treatment of diabetes [6,7]. In recent years, SGLT2 inhibitors mainly include dapagliflozin, ipragliflozin, empagliflozin, and canagliflozin, and dapagliflozin is the most selective, reversible, and orally active inhibitor of SGLT2 for wide clinical use. Multiple clinical trials showed that dapagliflozin can stably decrease blood glucose, reduce the risk of hypoglycemia, and improve glucose tolerance, insulin resistance, and glycated hemoglobin levels [8,9].

Renal cell carcinoma (RCC) accounts for approximately 2% of all human malignancies; it affects more men than women, with a ratio of 2: 1 [10]. RCC is classified into several types: clear cell type, granular cell type, mixture cellularity, and undifferentiated cell type [10]. Based on literature searches, multiple pathogenic factors are related with RCC malignancy, including obesity, eating habits, smoking, drinking, and long-term exposure to radioactivity [11]. Surgery, radiotherapy, chemotherapy, and immunotherapy are by far most commonly used therapeutic methods of RCC, but they have a small curative effect and a negative impact on RCC patients [12]. Therefore, it is urgent to seek efficient and low-toxicity anticancer drugs and treatment for RCC.

SGLT2 is widely located on the surface of tumor cells, such as hepatocellular carcinoma, colorectal cancer cells, breast cancer cells, and esophageal cancer cells. The principal metabolic pathway and the main energy source of tumor cells are glycolysis and glucose, respectively, and SGLT2 plays an important role in glycolysis and glucose in tumor cells [13-15]. However, there is little known about the relationship between SGLT2 and renal cancer cells.

Therefore, it is reasonable to hypothesize that increasing the SGLT2 expression level might provide a therapeutic target for RCC. The aim of this study was to elucidate the effects of dapagliflozin in RCC cell lines *in vitro* and *in vivo*.

Material and Methods

Cell lines and culture

ACHN, A498, and CaKi-1 human renal cell carcinoma (RCC) cell lines, and HK-2 (Human Glandular KalliKrein-2) cells were all purchased from the American Type Culture Collection (ATCC, VA, USA). All the cells were cultured in DMEM high-glucose medium (Gibco, New York, USA) supplemented with 10% FBS (Gibco, New York, USA), streptomycin (50 units/mL) and penicillin (100 units/mL) at 37°C in a humidified incubator containing v/v 5% CO_2.

MTT assays

Cell proliferation assays were carried out by using 3-[4,5-Dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Beyotime Biotechnology, China). Briefly, 2000 cells/well were seeded in triplicate in 96-well plates. The cells were treated with different concentrations of dapagliflozin (1, 2 and 4 µM) for 24 h, 48 h, and 72 h (n=3). We added 20 µL of MTT stock solution (MTT reagent was prepared at 5 mg/ml in PBS) per well and incubated it at 37°C for 4 h, and the formative formazan crystals were dissolved in 150 µL DMSO. The absorbance of samples at 450 nm was measured using a Multiscan Spectrum device (Biotek, USA). This experiment was carried out 3 times.

Cell cycle analysis

Cell cycle analysis was performed by flow cytometry as described in a previous study [16]. Firstly, 2×10^5 cells were seeded in a 6-well culture plate for 24-h culturing. Then, 2 µM dapagliflozin was added into cells. After co-culturing for 48 h, the cells were trypsinized, and washed with PBS twice. Finally, cells were stained with propidium iodide (Sigma-Aldrich) according to the manufacturer's instructions. The cell cycle distribution was analyzed by MultiCycle software. The experiment was performed 3 times.

Apoptosis assays

Cell apoptosis was quantified by Annexin V-fluorescein isothiocyanate/propidium iodide (Sigma-Aldrich) staining. After treatment with 2 μM dapagliflozin for 48 h, CaKi-1 cells were washed twice with PBS, then cells were collected and re-suspended with binding buffer. Annexin V-fluorescein isothiocyanate and propidium iodide were added for incubation in the dark for 15 min. Finally, the cells were analyzed by flow cytometry (Beckman Coulter, USA). The experiment was performed 3 times independently.

RNA extraction and quantitative real-time reverse transcription PCR (qRT-PCR)

Total RNA samples were extracted from cells and tumors by using the TRIzol reagent (Invitrogen) following the manufacturer's protocol. The extracted RNA samples were determined by the 260/280 nm ratio. PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa) was used for qRT-PCR. The primers for SGLT2: 5'-ACGCCTGATTCCCGAGTT-3', 5'-GCGGTGGAGGTGCTTTCT-3' and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5'-TGTTCGTCATGGGTGTGAAC-3', 5'-ATGGCATGGACTGTGGTCAT-3', GAPDH gene was selected as the endogenous gene. All experiments were performed in duplicate and repeated twice. The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold changes of the SGLT2 gene.

Western blot analysis

Total proteins were extracted from the HK-2, ACHN, A498, and CaKi-1 cells by adding RIPA buffer (Beyotime Biotechnology, China) on ice. The concentrations of the extracted proteins were quantified by BCA Protein Quantitation Kit (Beyotime Biotechnology, China), and then CaKi-1 cells were incubated with a 2-μM concentration of dapagliflozin for 48 h before being extracted. In animal experiments, at 7, 14, and 21 days after treatment, 6 mice from each group were sacrificed. The same amount of tumor samples was mechanically homogenized in tissue total protein lysis buffer (X-Y Biotechnology, Ltd) and centrifuged at 12 000 g for 5 min at 4°C. The supernatant was collected and quantified by use of the BCA Protein Quantitation Kit. Briefly, proteins were subjected to 10% SDS-PAGE and then transferred to PVCF membranes (Bio-Rad). We used 5% nonfat milk to block the membranes for 1 h at room temperature. Then, the membranes were incubated with the SGLT2 antibodies (mouse anti-SGLT2 monoclonal antibody; 1: 500, Abcam) on ice overnight. The next day, mouse anti-GAPDH (1: 1000, Abcam) was used to incubate the membrane for 2 h. The protein expression was examined by enhanced chemiluminescence (ECL kit) reagents (Pierce), and imaged using ChemiDoc XRS (BIO-RAD, USA). The relative protein integrated density values were adjusted to the GAPDH gene as an internal control. The experiments were performed 3 times and the relative band intensity was detected using Image J software.

Glucose uptake

We seeded $1×10^4$ CaKi-1 cells in a 96-well culture plate for culturing overnight and treated them with the 1-, 2-, and 4-μM concentration of dapagliflozin for 48 h. Then, we incubated them with 100 μL of 2-NBDG 200 μg/mL glucose-free culture medium solution for 12 h. At the end of treatment, we centrifuged the plate for 5 min at 400×g at room temperature and aspirated the supernatant. We added 200 μL of Cell-Based Assay Buffer to each well and centrifuge the plate for 5 min at 400×g at room temperature and aspirated the supernatant, after which we added 100 μL of Cell-based Assay Buffer to each well and detected the fluorescein immediately (excitation/emission=485/535 nm) according to the illustration of the Glucose Uptake Cell-Based Assay Kit (Cayman, USA). The experiments were performed 3 times.

Immunofluorescence

We seeded $2×10^5$ cells in a 6-well culture plate for 24-h culturing and incubated it with 2 μM dapagliflozin for 48 h. Then, the CaKi-1 cells were fixed with 4% paraformaldehyde for 20 min and with 0.2% Triton X-100 for 10 min, followed by blocking with 5% goat serum albumin. The cells were incubated with anti-SGLT2 antibody (1: 100, Abcam) in a wet box overnight at 4°C. Then, cells were incubated with Goat Anti-Mouse IgG H&L (FITC) (1: 1000, Abcam) for 1 h at 37°C and counterstained by DAPI (1 μg/mL) for 15 min. Images of the cells were obtained using a laser scanning confocal microscope (Leica, TCS SP5, Germany) and the fluorescent intensity was quantified by Image-Pro Plus 6.0 software.

Xenograft model in nude mice

All experimental procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23, revised 1996) and were performed according to the institutional ethics guidelines for animal experiments. We subcutaneously injected $1×10^7$ CaKi-1 cells in the right region of 60 six-week-old athymic nude mice (30 males and 30 females). The mice were divided into a saline group (n=18) and a dapagliflozin group (n=18). After approximately 1 week, saline and dapagliflozin (1.3 mg/kg) were intravenously injected into tails of nude mice into which tumors were successfully transplanted. The tumor volumes were measured at days 3, 7, 10, 14, and 21 with calipers. The calculation of tumor volume was according with the formula: (L×W×H×π)/6, where L is the length, W is the width, and H is the height of the tumor. Six mice of each group were killed on days 7, 14, and 21. The SGLT2 expression of the tumors was tested through Western blot analysis and QPCR assays according to the methods described previously.
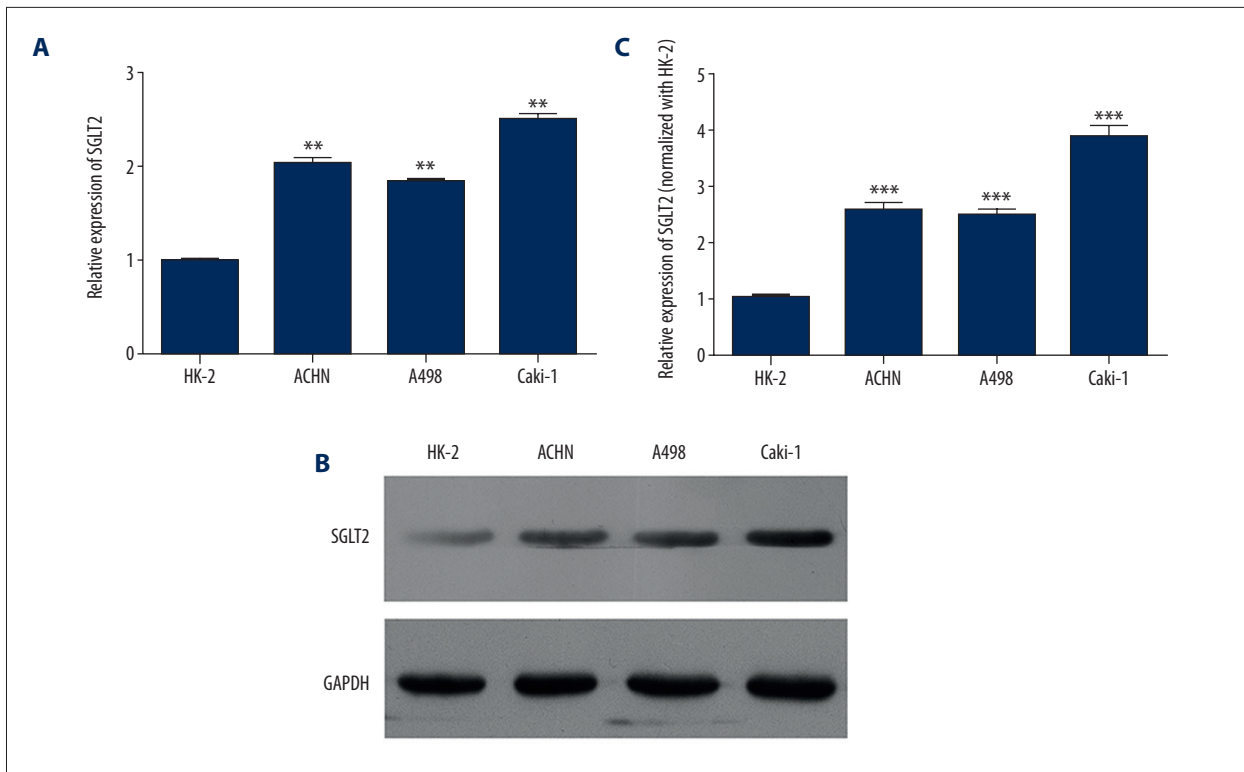
Figure 1. The relative mRNA and protein expression of SGLT2 on renal cells and effects of dapagliflozin on proliferation of normal cells and various renal cell carcinoma cell lines *in vitro*. (**A**) The relative mRNA expression of SGLT2 in HK-2, ACHN, A498 and CaKi-1 cells were examined by qRT-PCR assay. * $P<0.05$, ** $P<0.01$, n=3. (**B**) The protein expression of SGLT2 on HK-2, ACHN, A498, and CaKi-1 cells were detected by Western blot assay. n=3, ** $P<0.01$.

Hematoxylin and eosin staining

Firstly, the tumor tissues of the nude mice were fixed with 4% paraformaldehyde for 4 h, transferred to 70% ethanol, and embedded in paraffin. Secondly, the paraffin-embedded tissue was cut into 4-μm-thick sections. Before immunostaining, sections were dewaxed in xylene, dehydrated with different concentrations of ethanol, and washed with distilled water. Thirdly, the sections stained with hematoxylin and eosin (HE), rehydrated with increasing concentrations of ethanol, hyalinized with xylene, and mounted with neutral gum. Finally, the HE staining result was observed under a light microscope.

Immunohistochemistry

The paraffin-embedded tissues were prepared with the above method. After dewaxing and dehydration, the sections were immersed with 3% H_2O_2, washed with distilled water for 5 min×3 times, and incubated with 15% BSA by microwaving sections in sodium citrated buffer solution for 30 min. Then, the sections were incubated with anti-SGLT2 antibody (1: 100, Abcam) on ice overnight, followed by biotinylated anti-mouse antibody (1: 200) and horseradish peroxidase-labeled antibody. All the histological stains were repeated on 3 separate sections.

Statistical analysis

The experimental results are expressed as mean ± standard deviation unless otherwise noted; comparison among groups was performed using one-way ANOVA followed by Bonferroni analysis. *P* values of <0.05 were considered statistically significant.

Results

The relative expression of SGLT2 in different renal cells

The relative expression of SGLT2 was detected by qRT-PCR and Western blot assays. HK-2 cells are human normal renal cells, and ACHN, A498, and CaKi-1 cells are several common renal carcinoma cell lines. The results of qRT-PCR assay indicated that the expression level of SGLT2 in renal carcinoma cell lines was obviously higher when compared to HK-2 cells, and Caki-1 cells had a highest SGLT2 expression (Figure 1A, n=3, *P*<0.05). The data from Western blot assays were consistent with the qRT-PCR experiment results (Figure 1B, n=3, *P*<0.05).
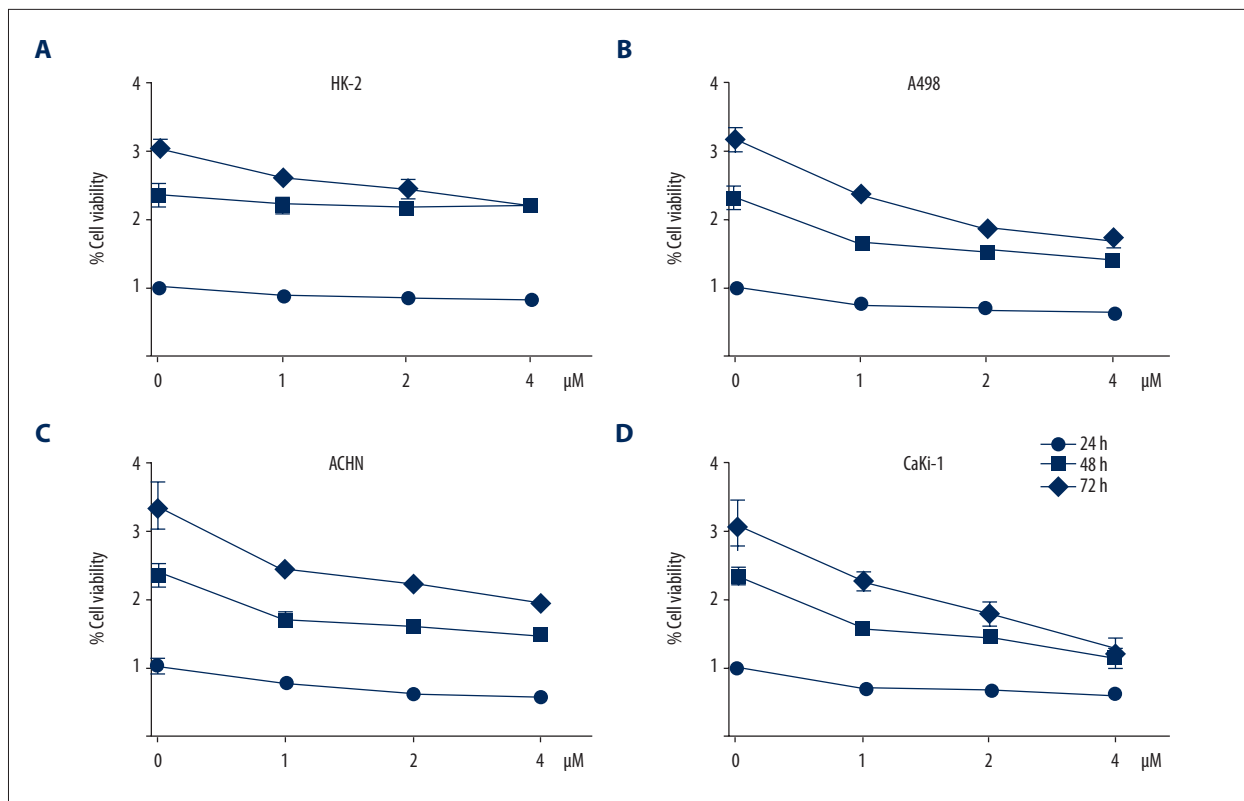
Figure 2. The cell growth was analyzed by MTT assay with 1-, 2-, and 4-μM dapagliflozin treatment for 24, 48, and 72 h: (**A**) HK-2, (**B**) A498, (**C**) ACHN and (D) CaKi-1 cells. n=3, * $P<0.05$, ** $P<0.01$.

Effects of dapagliflozin on proliferation of renal cell carcinoma cell lines *in vitro*

The effects of dapagliflozin on proliferation of renal carcinoma cell lines were investigated by MTT assays. As shown in Figure 2A–2D, when HK-2, A498, ACHN, and CaKi-1 cells were treated with different concentrations of dapagliflozin (1, 2, and 4 μM) at 24, h, 48 h, and 72 h, dagliflozin significantly inhibited the cell growth in a dose- and time-dependent manner. In addition, dapagliflozin is more sensitive to ACHN, A498, and CaKi-1 than HK-2 cells (n=3, $P<0.05$). Therefore, CaKi-1 cells were chosen to further study the antitumor activity of dapagliflozin.

Effects of dapagliflozin on cell cycle and apoptosis of CaKi-1 cells

To study the underlying mechanisms of dapagliflozin on inhibiting proliferation of renal carcinoma cells, Caki cells were treated with dapagliflozin 2 μM for 48 h, and the cell cycle and apoptosis were analyzed by flow cytometry. As the results show in Figure 3A, cells in G1 phase increased by 1.49-fold and cells in S phase decreased to 54.4% that of the control group, indicating dapagliflozin induced G1 phase arrest in Caki-1cells (n=3, $P<0.05$). Furthermore, we found dapagliflozin treatment increased the cellular apoptosis rate by 1.89-fold

compared to the control group, including early apoptosis and late apoptosis (n=3, $P<0.05$) (Figure 3B).

Effects of dapagliflozin on cell glucose uptake and the SGLT2 protein expression in CaKi-1 cells

Because Dapagliflozin is known as a special SGLT2 inhibitor, we investigated the protein expression of SGLT2 by Western blot assay after CaKi-1 cells were incubated with 2 μM dapagliflozin for 48 h. We found that dapagliflozin inhibited the SGLT2 expression (Figure 4A). The fluorescence intensity of SGLT2 was significantly reduced in the dapagliflozin group, consistent with the result of Western blot assay (Figure 4B, 4C). To investigate the functions of dapagliflozin further, we evaluated the effects of co-culturing CaKi-1 cells and glucose. As shown in Figure 4D, treatment with dapagliflozin (2 μM and 4 μM) resulted in a significantly reduced ratio at 25% and 43% in glucose content compared to the control group (n=3, $P<0.05$).

Effects of dapagliflozin on tumor growth *in vivo*

Furthermore, the function of dapagliflozin on the growth of CaKi-1 cells was examined in tumor-bearing nude mice. Saline and dapagliflozin (1.3 mg/kg) were injected intravenously into tails of nude mice, and the tumor sizes were recorded at
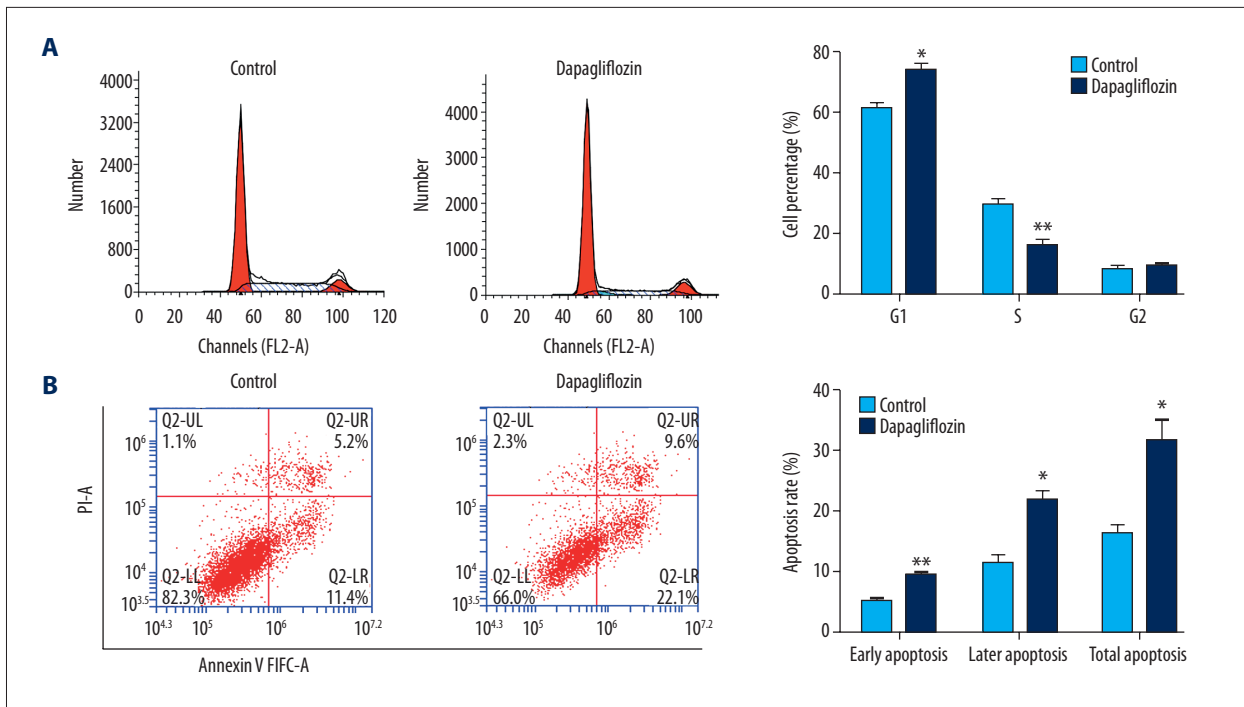
Figure 3. Effects of dapagliflozin on cell cycle and apoptosis. (**A**) Flow cytometry analysis of the effect of dapagliflozin on cell cycle of CaKi-1 cells. * *P*<0.05, ** *P*<0.01, n=3. (**B**) Flow cytometry analysis the effect of dapagliflozin on cell apoptosis of CaKi-1 cells. n=3, * *P*<0.05, ** *P*<0.01.

different times. As Figure 5A shows, the tumors treated with dapagliflozin were smaller than in the saline group at 21 days. Tumor volumes decreased after injecting dapagliflozin compared to the saline group (n=6, *P*<0.05), as shown in Figure 5B, 5C, indicating dapagliflozin inhibited the tumor growth. The obtained tumors were used to further study the SGLT2 expression of tumors by Western blot and qRT-PCR. As shown in Figure 5D-F, dapagliflozin significantly reduced the SGLT2 mRNA and protein expression compared to the control group at 7, 14, and 21 days (n=6, *P*<0.05).

HE staining and immunohistochemistry methods were used to observe the pathological changes of the tumor tissues of nude mice. As show in Figure 5G, the HE staining results showed a loose arrangement and a large necrotic region in the dapagliflozin group compared with the saline group. In addition, the immunohistochemistry results of SGLT2 in the saline group exerted predominantly cytoplasm staining, and we observed a marked reduction in the dapagliflozin group (Figure 5H).

Discussion

Highly selective inhibitors of the sodium glucose co-transporter 2 (SGLT2) are showing promise as useful agents adding to the current therapeutic options in type 2 diabetes mellitus [17]. Dapagliflozin is the first SGLT2 inhibitor demonstrated

to clearly reduce hemoglobin A_{1c} (HbA_{1c}) and fasting plasma glucose (FPG) in clinical therapy [18,19]. SGLT2 gene expression profiling demonstrated ubiquitous distribution in kidney, colon, and liver [20]. Therefore, it is critical to better understand whether renal cell carcinoma has a relationship with SGLT2. qRT-PCR and Western blot assay showed that the relative mRNA and protein SGLT2 in renal cell carcinoma were higher than in normal renal cells (Figure 1A, 1B).

However, the function of dapagliflozin in renal cell carcinoma remains unclear. Dapagliflozin is new therapeutic class in the marketplace. We observed that dapagliflozin shows a cytotoxic effect on human renal cell carcinoma, and exhibits hypotoxicity to normal human renal cells in culture (Figure 2A, 2D). In the current study, we also found that dapagliflozin regulated cell cycle and apoptosis in CaKi-1 cells (Figure 3). These results suggest that dapagliflozin is involved in the regulation of the malignant behavior of CaKi-1 cells.

Glucose plays an important role in maintaining the energy metabolism of tumor cells, promoting new angiogenesis, tumor proliferation, invasiveness, and metastasis [21,22]. To investigate this further, the effects of dapagliflozin on glucose uptake were evaluated. Figure 4D shows dapagliflozin reduced the glucose uptake in CaKi-1 cells in dose-dependent manners. We also found decreased expression of SGLT2 in the group with dapagliflozin treatment (Figure 4A–4C), which suggests that
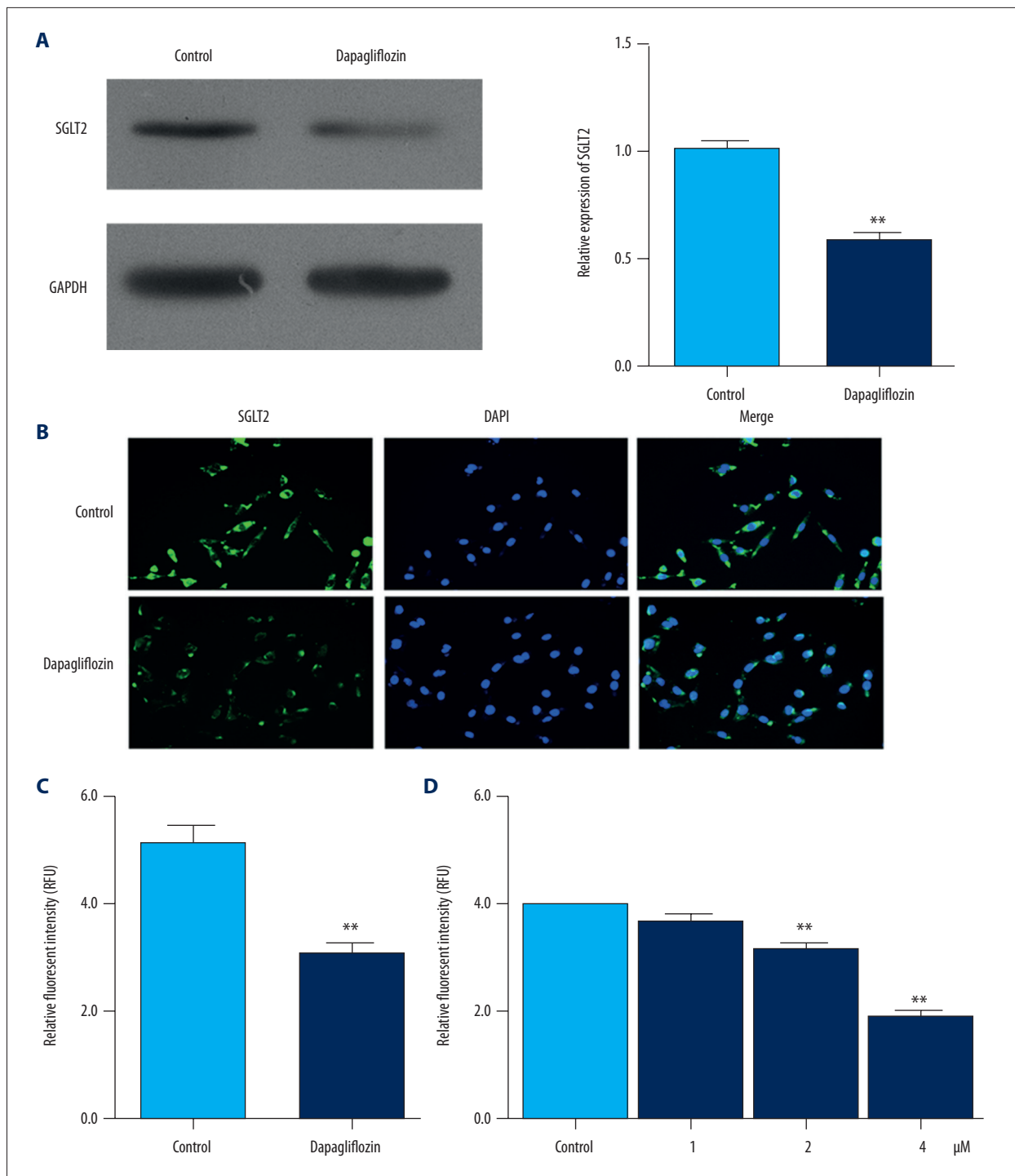
Figure 4. Effects of dapagliflozin on cell glucose uptake and SGLT2 protein expression. (**A**) The protein expression of SGLT2 was determined by Western blot assay after dapagliflozin treatment and the intensity was tested by Image J software. (**B, C**) Immunofluorescence of SGLT2 in CaKi-1 cells. FITC-labeled secondary antibodies were used (green fluorescence), and nuclei were stained by DAPI (200× magnification). The fluorescent intensity was tested by Image-Pro Plus 6.0 software. (**D**) After dapagliflozin treatment, CaKi-1 cells were incubated with 12 mM glucose for 24 h and the glucose uptake was measured by liquid scintillation spectrometer, n=3, * $P<0.05$, ** $P<0.01$.
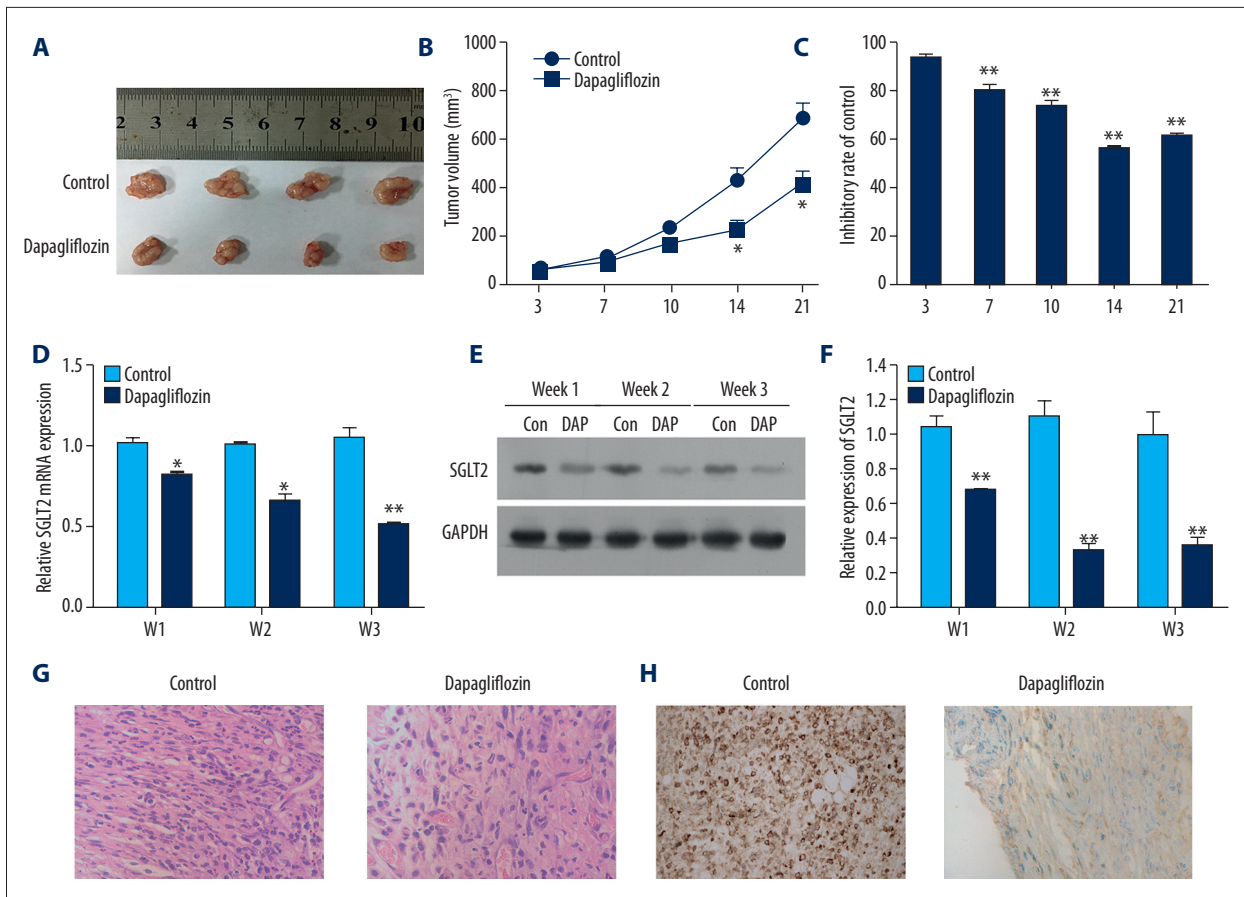
Figure 5. Effects of dapagliflozin on tumor growth *in vivo* and pathology changes in tumor tissue of nude mice induced by dapagliflozin. The stable expressing CaKi-1 cells were used for *in vivo* research. (**A**) Tumor bodies after being treated with saline or dapagliflozin for 21 days. (**B**) Tumor volume was calculated at 3, 7, 10, 14, and 21 days after injection. (**C**) Inhibition rate of dapagliflozin compared to saline group. n=6, * *P*<0.05, ** *P*<0.01 *vs.* saline group. (**D**) The SGLT2 mRNA expression of tumors were examined for 3 weeks by qRT-PCR assay. (E-F) The SGLT2 expression of tumors was examined for 3 weeks by Western blot assay. n=6, * *P*<0.05, ** *P*<0.01. (**G**) HE staining and (**H**) SGLT2 immunohistochemistry staining from nude mice after being treated with saline or dapagliflozin for 21 days. Original magnification: 400×.

dapagliflozin can inhibit the protein expression of SGLT2 associated with glucose metabolism. All these results suggest that dapagliflozin can decrease the malignant behavior of CaKi-1 cells via down-regulating SGLT2. The *in vivo* experiment also indicated that the nude mice with dapagliflozin treatment produced significantly smaller tumors and reduced SGLT2 expression (Figure 5A–5F). These data suggest dapagliflozin functions as a tumor suppressor in renal carcinoma. In the dapagliflozin-treated mice, we found dapagliflozin caused a significant increase in tumor necrosis and a less weakly positive expression of SGLT2 (Figure 5G, 5H), suggesting that dapagliflozin may have an effect on tumor necrosis.

Conclusions

These results show that dapagliflozin as a SGLT2 inhibitor can reduce the viability of renal carcinoma cell lines, regulate the cell cycle and apoptosis, and also reduce tumor volume. We speculate that the functional expression of SGLT2 in renal carcinoma brings new diagnostic and therapeutic possibilities for renal carcinoma.

Conflict of interests

The authors have declared that no competing interests exist.

References:

1. Liu S, Wang W, Zhang J et al: Prevalence of diabetes and impaired fasting glucose in Chinese adults, China National Nutrition and Health Survey, 2002. Prev Chronic Dis, 2011; 8(1): A13

2. Report on chronic disease risk factor surveillance in China 2010. National Center for Chronic and Noncommunicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, 2012

3. Kuecker CM, Vivian EM: Patient considerations in type 2 diabetes – role of combination dapagliflozin-metformin XR. Diabetes Metab Syndr Obes, 2016; 9: 25–35

4. Dzhambov AM, Dimitrova DD: Exposures to road traffic, noise, and air pollution as risk factors for type 2 diabetes: A feasibility study in Bulgaria. Noise Health, 2016; 18(82): 133–42

5. Korkmaz-Icöz S, Al Said S, Radovits T, Li S et al: Oral treatment with a zinc complex of acetylsalicylic acid prevents diabetic cardiomyopathy in a rat model of type-2 diabetes: Activation of the Akt pathway. Cardiovasc Diabetol, 2016; 15(1): 75

6. Shyangdan DS, Uthman OA, Waugh N: SGLT-2 receptor inhibitors for treating patients with type 2 diabetes mellitus: A systematic review and network meta-analysis. BMJ Open, 2016; 6(2): e009417

7. Cheng ST, Chen L, Li SY et al: The effects of empagliflozin, an SGLT2 inhibitor, on pancreatic beta-cell mass and glucose homeostasis in Type 1 Diabetes. PLoS One, 2016; 11(1): e0147391

8. May M, Schindler C: Clinically and pharmacologically relevant interactions of antidiabetic drugs. Ther Adv Endocrinol Metab, 2016; 7(2): 69–83

9. Reilly TP, Graziano MJ, Janovitz EB et al: Carcinogenicity risk assessment supports the chronic safety of dapagliflozin, an inhibitor of sodium-glucose co-transporter 2, in the treatment of type 2 diabetes mellitus.Diabetes Ther, 2014; 5(1): 73–96

10. Rini BI, Campbell SC, Escudier B: Renal cell carcinoma. Lancet, 2009; 373(9669): 1119–32

11. Ray RP, Mahapatra RS, Khullar S et al: Clinical characteristics of renal cell carcinoma: Five years review from a tertiary hospital in Eastern India. Indian J Cancer, 2016; 53(1): 114–17

12. Sun J, Signoretti S, Zhu Q, Marasco WA: Human anti-CAIX antibodies mediate immune cell inhibition of renal cell carcinoma *in vitro* and in a humanized mouse model *in vivo*. Mol Cancer, 2015, 14: 119

13. Saito T, Okada S, Yamada E et al: Effect of dapagliflozin on colon cancer cell. Endocr J, 2015; 62(12): 1133–37

14. Scafoglio C, Hirayama BA, Kepe V et al: Functional expression of sodium-glucose transporters in cancer. Proc Natl Acad Sci USA, 2015; 112(30): E4111–19

15. Lin HW, Tseng CH: A Review on the relationship between SGLT2 inhibitors and cancer. Int J Endocrinol, 2014; 2014: 719578

16. Dong L, Lv H, Li W et al: Co-expression of PD-L1 and p-AKT is associated with poor prognosis in diffuse large B-cell lymphoma via PD-1/PD-L1 axis activating intracellular AKT/mTOR pathway in tumor cells. Oncotarget, 2016; 7(22): 33350–62

17. Scafoglio C, Hirayama BA, Kepe V et al: Functional expression of sodium-glucose transporters in cancer. Proc Natl Acad Sci USA, 2015; 112(30): E4111–19

18. Singh AK, Singh R: Sodium-glucose co-transporter-2 inhibitors and dipeptidyl peptidase-4 inhibitors combination therapy in type 2 diabetes: A systematic review of current evidence. Indian J Endocrinol Metab, 2016; 20(2): 245–53

19. Skolnik N, Bonnes H, Yeh H, Katz A: Dapagliflozin in the treatment of patients with type 2 diabetes presenting with high baseline A1C. Postgrad Med, 2016; 128(4): 356–63

20. Chen J, Williams S, Ho S et al: Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. Diabetes Ther, 2010; 1(2): 57–92

21. Yang R, Cao L, Gasa R et al: Glycogen-targeting subunits and glucokinase differentially affect pathways of glycogen metabolism and their regulation in hepatocytes. J Biol Chem, 2002; 277(2): 1514–23

22. Chowdhury B, Porter EG, Stewart JC et al: PBRM1 Regulates the expression of genes involved in metabolism and cell adhesion in renal clear cell carcinoma. PLoS One, 2016; 11(4): e0153718