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Short Communication

Determining the effects of SGLT2 inhibitor on breast cancer cell migration and clonogenicity – a pilot study

Vincent Lučanský[1], Ivana Baranová[1,2], Iryna Samec[3], Martin Jozef Péč[4], Renata Péčová[1] and Marek Samec[5]

[1] *Department of Pathological Physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*
[2] *Biobank for Cancer and Rare Diseases, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*
[3] *Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*
[4] *1st Department of Internal Medicine, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava and University Hospital Martin, Martin, Slovakia*
[5] *Department of Medical Biology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*

Abstract. Breast cancer remains one of the most diagnosed and lethal cancers, with increased incidence in developing countries. Empagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, is an effective and well-tolerated antidiabetic drug for managing and treating type 2 diabetes mellitus. Moreover, it can reduce the risk of death from cardiovascular causes and hospitalizations for heart failure. Recent data indicate that empagliflozin has antioxidant and anti-inflammatory effects mediated by modulating various signaling pathways. Molecular and cellular analysis revealed that SGLT2 is expressed in different types of cancer, including breast, cervical, and lung. We analyzed the potential anticancer activities of empagliflozin in the 4T1 breast cancer cell line. To assess the effect of the SGLT2 inhibitor on the migration and clonogenicity of cancer cells, we used the wound healing assay and the colony formation assay. The acquired data showed some trends; however, no significant results were obtained.

Key words: Breast cancer — Clonogenicity — Migration — Empagliflozin

Highlights:

- Empagliflozin, an SGLT2 inhibitor, demonstrates potential anticancer effects.
- Using wound healing assay and colony formation assay, we tested the effect of empagliflozin on the migratory and clonogenic potential of 4T1 cells.
- Treatment with 1 μM empagliflozin caused a non-significant reduction in the clonogenic potential of 4T1 cells, and a slight increase in cell migration was observed.

Oncological diseases are the leading cause of premature death, resulting in approximately 10 million deaths worldwide in 2022. Cancer often affects multiple organs due to uncontrolled cell proliferation, migration, and invasive behavior. To fully understand the pathogenesis of cancer is of paramount importance due to the constantly growing

Correspondence to: Renata Péčová, Department of Pathological Physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, 036 01, Slovakia
E-mail: renata.pecova@uniba.sk

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number of cases worldwide and often limited treatment options, which contribute to low overall and relapse-free survival rates (Yin et al. 2021; Koya and Ibrahim 2025).

Breast cancer is the most common type of cancer in women, with an estimated 2.3 million new cases diagnosed globally each year (Łukasiewicz et al. 2021). Breast cancer treatment requires a multidisciplinary approach that takes into account tumor burden and the presence of prognostic and predictive molecular markers. Early-stage breast cancer is typically treated surgically in combination with radiotherapy or mastectomy alone. In the case of metastatic breast cancer, the goal of treatment is to stop or slow the growth of the tumor and prolong survival and maintain the quality of the patient´s life using agents such as tamoxifen, trastuzumab, paclitaxel, or doxorubicin, depending on the tumor type (Wang et al. 2023; Xiong et al. 2025). The need and demand for new drugs that block tumor growth is, therefore, understandable.

Empagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, is a highly effective and well-tolerated antidiabetic agent of the C-glucoside family (Shafiq et al. 2024). In addition to its hypoglycemic effect, it also exhibits several other beneficial properties, including hypotensive and cardioprotective effects. It has anti-inflammatory and antioxidant potential in diabetic nephropathy (Wanner et al. 2016; Liu et al. 2020). Several studies also indicate its antitumor effects (Flausino et al. 2025; Pandey et al. 2025). The SGLT2 receptor is expressed in various tumor cell lines such as pancreatic tumors, prostate tumors, breast tumors, and glioblastomas (Wright 2020; Wu et al. 2023). One of the suggested molecular mechanisms of action of empagliflozin is the inhibition of glucose reabsorption in the renal tubules, leading to a decrease in glucose availability for tumor cell metabolism and, consequently, the suppression of their growth and proliferation. However, the specific mode of action on tumor cells remains unclear and requires further investigation (Dutka et al. 2022).

In this pilot study, we aim to test and optimize the laboratory protocols for the methods allowing to determine the clonogenicity and migratory potential of tumor cells either treated or untreated with empagliflozin. For this purpose, we utilized the triple-negative breast carcinoma cell line 4T1 (ATCC® CRL-2539™) derived from a mouse tumor. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Cat. No. D5796) supplemented with 10% FBS (Gibco), 1% Non-Essential Amino Acids Solution (NEAA) (Gibco), and 1% Antibiotic Antimycotic Solution (Anti-Anti) (Gibco). Cells were incubated in an incubator with a 5% CO_2 atmosphere at 37°C.

To perform the wound healing assay, 4T1 cells were trypsinized, conted, and then seeded in a 24-well plate at a density of 5×10^5. After 48 hours of incubation at 37°C, monolayers were scratched, creating a wound without the cells. The scratch was made with a sterile micropipette tip (200 µl) held at approximately a 90-degree angle to ensure a uniform width of the gap. The medium was then removed, and the wells were washed twice with PBS (500 µl) to remove loose cells. Empagliflozin (0.5 mM stock solution in DMSO) was added to DMEM medium supplemented with 10% FBS, 1% NEAA, and 1% Anti-Anti to achieve final concentrations of 1 µM and 10 µM. Subsequently, 500 µl of the prepared medium with respective empagliflozin concentrations was added to the wells. The experiment included three experimental groups: 1) control group with the addition of DMSO; 2) test group with a concentration of 1 µM empagliflozin; 3) test group with a concentration of 10 µM empagliflozin. To evaluate the effects of empagliflozin, experiments were performed in three biological replicates. Further, the wound area was photographed using an inverted confocal microscope (Zeiss LSM 880 Scanning Confocal Microscope) before the addition of the drug, as well as after 4, 8, and 24 hours, using a 10× magnification objective. To ensure a consistent field of view for each scan, reference marks were drawn on the lower outer part of the plate wells using an ultra-fine permanent marker. These marks were placed outside the scan area. In each well, the area of the scratch below the reference line was photographed. Images were then analyzed using ImageJ2 software, version 2.16.0/Fiji, distributed under the GNU General Public License version 3 (GPLv3). The percentage of wound closure (%) was quantified based on the percentual change in the normalized measured area divided by the original open area according to the formula: Wound closure (%) = $[(A_0 - A_t)/A_0] \times 100$, where A_0 represents the wound area at time 0 and A_t the area after the selected incubation time (t).

To perform a colony formation assay, a suspension of 4T1 cells was seeded in six-well plates at a concentration of 1×10^3 cells *per* well, and the cells were allowed to adhere overnight. After adherence, the culture medium was replaced (1 ml/well) either with medium containing 1 µM empagliflozin or with the medium supplemented with a corresponding concentration of DMSO as a negative control. Subsequently, the cells were incubated in an atmosphere of 5% CO_2 at 37°C for 6 days, and growth in all six-well plates of a given cell line was terminated simultaneously. Fixation was performed using 70% ethanol, and staining was done with 0.5% crystal violet (Sigma Aldrich, Taufkirchen, Germany). Colonies with ≥ 50 cells were analyzed using ImageJ/Fiji software.

Comparisons between groups were performed using the Mann-Whitney test and analysis of variance (ANOVA), followed by multiple comparisons using Dunnett's test. Three data sets were evaluated, with DMSO-treated cells serving as a control. Data are presented as mean ± standard deviation (SD), and a p value < 0.05 was considered statistically significant. Graphs and statistical analyses were performed using GraphPad Prism software, version 5.0 (San Diego, CA, USA).

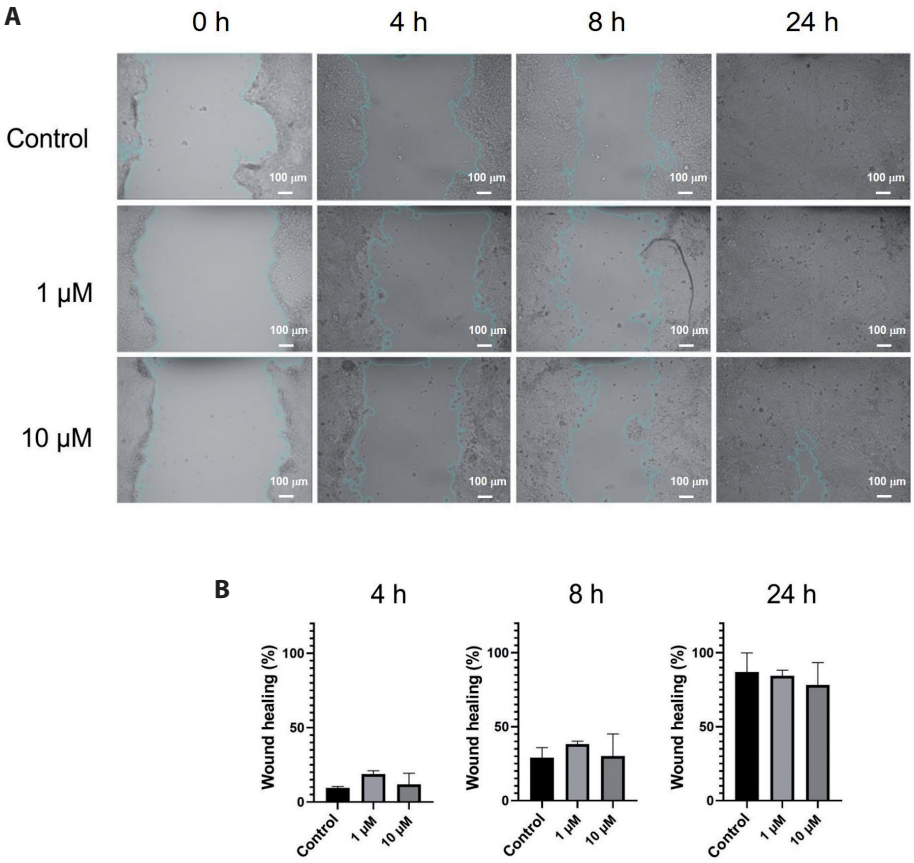


Figure 1. A. Comparison of wound closure in different groups treated with 1 mM and 10 mM empagliflozin or a negative control at time intervals after 4, 8, and 24 hours. **B.** Quantification of the wound closure at time intervals after 4, 8, and 24 hours.

We observed an increased wound closure rate compared to the control group after 4 and 8 hours in the group treated with empagliflozin at a concentration of 1 μM. At a concentration of 10 μM, the changes in the wound closure rate were only minimal. After 24 hours, a change in the trend was observed. The most pronounced wound closure was observed in the control group, whereas the wound closure process in the groups treated with empagliflozin was slowed down. However, the differences were not statistically significant. (Fig. 1).

Further, we assessed the ability of tumor cells to form colonies, i.e., cell clones originating from a single original cell. To quantify this phenomenon, we employed a clonogenic assay, which enables the visualization and subsequent counting of individual colonies. The empagliflozin-treated group showed a slightly lower number of colonies (on average 137.6 ± 3.29) compared to the control group (average 149.6 ± 5.55), which was cultured in the absence of SGLT2 inhibitor ($p = 0.0585$). However, observed differences were not statistically significant. (Fig. 2).

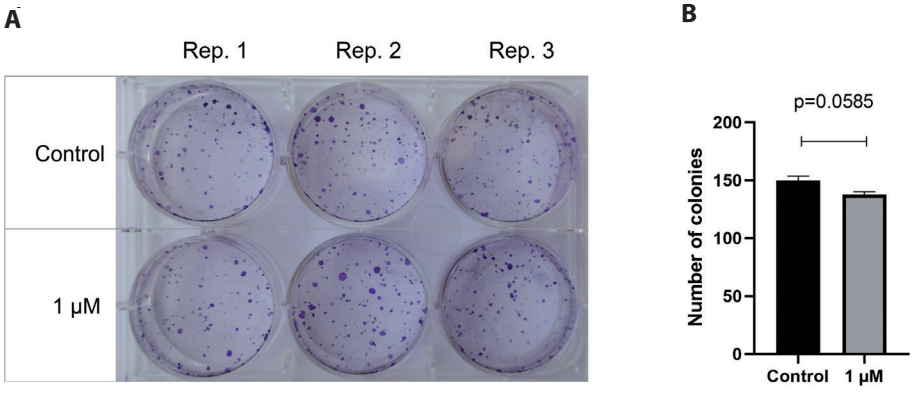


Figure 2. A. Clonogenic assay showing the long-term effect (5 days) of 1 μM empagliflozin (lower wells) on 4T1 tumor cell lines compared to the untreated control group (upper wells). The experiment was performed in three biological replicas. **B.** Quantitative comparison of colony numbers between tumor cells after the addition of 1 μM empagliflozin and the control without therapy. Rep., replica.

The SGLT2 inhibitor empagliflozin is a proven and effective treatment for type 2 diabetes (Dutka et al. 2022). Due to its anti-inflammatory effects, it can reduce the risk of complications associated with diabetes (Hattori 2018; García-Ropero et al. 2019). It also exhibits significant cardioprotective properties, making it a promising therapeutic option for patients with heart failure and other cardiovascular diseases (Jiang et al. 2022; Modzelewski et al. 2024). Empagliflozin also exhibits anticancer activity in several types of cancer, including the suppression of proliferation, migration, and invasiveness, as well as the induction of apoptosis in tumor cells. In combination with some chemotherapeutic agents or radiotherapy, it can increase therapeutic efficacy while reducing the incidence of adverse effects (Wu et al. 2023).

In our study, we aimed to demonstrate the regulatory effect of empagliflozin on the migration of metastatic 4T1 tumor cells. Using a wound healing assay, we were unable to demonstrate a statistically significant alteration in cell migration between groups treated with empagliflozin and the control. Although some effect was observed after 4 and 8 hours of cell culture with 1 μM empagliflozin, after 24 hours, the trend was reversed, with the control group showing the highest migration rate, which was reflected in faster wound closure. This phenomenon may be a consequence of the limited effect of empagliflozin over time, possibly due to its degradation in the culture medium, or a change in the cell phenotype from migratory to proliferative (Gunnarsson et al. 2020; Pathak et al. 2021). When using a higher concentration of empagliflozin, we did not observe any significant changes in the wound closure rate. This result may be associated with off-target effects of empagliflozin at higher concentrations or with the overload of signaling pathways that empagliflozin affects (Madonna et al. 2020; Mihaljević et al. 2022). This could explain the minimal change in cell migration between the higher concentration group and the control.

Further, we assessed the ability of tumor cells to form colonies using a clonogenic assay. This method is standardly used to assess the ability of individual cells to survive and proliferate after exposure to various compounds. Based on the wound healing assay outcome, we chose a concentration of 1 μM. Experimental results showed a slightly reduced number of viable colonies in the empagliflozin-treated group compared to the control; however, the outcome was not significant.

Previous studies have identified several mechanisms through which SGLT2 inhibitors may affect tumor progression. These include inhibition of β-catenin and EGFR, or activation of AMPK, observed in association with canagliflozin and dapagliflozin usage, which led to mTOR inhibition in breast cancer models. A decrease in the expression of SREBP1 and SCD1 genes was also noted in breast cancer and hepatocellular carcinoma cells (Dutka et al. 2022). Interestingly, in addition to tumor-suppressive effects, some clinical studies have also reported signals of potential pro-tumor effects of SGLT2 inhibitors. Specifically, there was a slightly increased incidence of bladder cancer in men and breast cancer in women, as well as an increased risk of renal cell carcinoma in patients treated with this group of drugs (Inose et al. 2025; Pandey et al. 2025).

Although our pilot study did not yield any statistically significant findings and only indicated some borderline outcomes, it nevertheless revealed observable trends. A possible explanation for the low efficacy of empagliflozin treatment may lie in the fact that the 4T1 cell line exhibits relatively low expression of the SGLT2 receptor. In general, considerable heterogeneity between various tumor cells and tumor types needs to be taken into account. Both the wound healing assay and the clonogenic assay demonstrated potential for further research on a diverse spectrum of cancer cell lines in the following preclinical studies.

Conflicts of interest. Authors declare no conflicts of interest.

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