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Empagliflozin Inhibits OSCC Proliferation and Migration by Suppressing SLC2A3 and NLRP3

Empagliflozin Oral Squamöz Hücreli Karsinomada SLC2A3 ve NLRP3 İfadesini Baskılayarak Proliferasyonu ve Migrasyonu Engeller

Gülçin TEZCAN

Bursa Uludağ University Faculty of Dentistry, Department of Fundamental Sciences, Bursa, Türkiye

ABSTRACT

Aim: High glucose and inflammation facilitate oral squamous cell carcinoma (OSCC) progression. The glucose transporter solute carrier family 2 member 3 (SLC2A3) and the nucleotide-binding oligomerization domain, leucine-rich repeat-containing family pyrin domain containing 3 (NLRP3), contribute to chronic inflammation and poor prognosis. This study investigates the regulatory effects of the antidiabetic drug empagliflozin (emp) on SLC2A3 and NLRP3 in OSCC and their impact on cancer cell growth.

Materials and Methods: Human OSCC cell lines, SCCL-MT1 and UPSI-SCC-131, were cultured in Dulbecco's Modified Eagle Medium with supplements. Emp, lipopolysaccharide, and adenosine triphosphate were used for treatments. Real-time polymerase chain reaction quantified SLC2A3 and NLRP3 expressions. Enzyme-linked immunosorbent assay measured interleukin-1beta (IL-1β) levels, and cell proliferation was assessed using the xCELLigence system. Migration was evaluated via a scratch wound assay. Protein-protein interactions were predicted using the STRING database. Data analysis was conducted using GraphPad Prism.

Results: Emp, an SGLT2 inhibitor, significantly reduced SLC2A3 ($p<0.0001$, $p=0.0002$; respectively) and NLRP3 expression ($p=0.0008$; $p=0.0006$; respectively), leading to decreased IL-1β release ($p=0.0190$, $p<0.0001$; respectively), proliferation ($p=0.024$; $p<0.0001$; respectively), and migration ($p=0.0021$; $p=0.0004$, respectively) in SCCL-MT1 and UPSI-SCC131 OSCC cell lines. These findings suggest emp's potential as a therapeutic agent for OSCC by targeting glucose metabolism and inflammation.

Conclusion: These findings suggest that emp effectively modulates glucose metabolism and inflammation in OSCC by inhibiting SLC2A3 and NLRP3 expression and IL-1β release, thereby reducing cancer cell proliferation and migration, highlighting its possible role as a treatment option for managing OSCC progression.

Keywords: Oral squamous cell carcinoma, SLC2A3, NLRP3, empagliflozin

ÖZ

Amaç: Oral squamöz hücreli karsinom (OSCC), ilerlemesi için yüksek glikoz metabolizması ve enflamasyona bağımlıdır. Glukoz taşıyıcı çözücü taşıyıcı ailesi 2 üyesi 3 (SLC2A3), kötü prognoz ile ilişkilidir, nükleotid bağlayıcı oligomerleşme bölgesi, lösin-zengin tekrar içeren aile pirin domain içeren 3 (NLRP3) ise kronik iltihaplanmaya katkı sağlar. Bu çalışma, antidiabetik ilaç empagliflozinin (emp) OSCC'de SLC2A3 ve NLRP3 üzerindeki düzenleyici etkilerini ve kanser hücresi büyümesi üzerindeki etkilerini araştırmaktadır.

Gereç ve Yöntem: İnsan OSCC hücre hatları SCCL-MT1 ve UPSI-SCC-131, suplementlerle zenginleştirilmiş Dulbecco's Modified Eagle Medium kültüre edilmiştir. Lipopolisakkarit maruziyeti ile enflamasyon modellenmiş ve adenozin trifosfat ile enflamazom aktivasyonu sağlanmıştır. Emp tedavi için kullanılmıştır. SLC2A3 ve NLRP3 ifadeleri, gerçek zamanlı polimeraz zincir reaksiyonu ile belirlenmiştir. Enzim bağlantılı immünosorbent testi, interlökin-1 beta (IL-1β) düzeylerini ölçerken, hücre proliferasyonu xCELLigence sistemi ile değerlendirilmiştir. Migrasyon sıyrık yara testi ile değerlendirilmiştir. Protein-protein etkileşimleri STRING veritabanı kullanılarak tahmin edilmiştir. İstatistiksel analiz GraphPad Prism ile yapılmıştır.

Address for Correspondence: Gülçin TEZCAN MD, Bursa Uludağ University Faculty of Dentistry, Department of Fundamental Sciences, Bursa, Türkiye
E-mail: gulcintezcan@uludag.edu.tr **ORCID ID:** orcid.org/0000-0002-5956-8755
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Bulgular: Emp, bir SGLT2 inhibitörü olarak, SCCL-MT1 ve UPSI-SCC131 OSCC hücre hatlarında SLC2A3 (sırasıyla p<0,0001, p=0,0002) ve NLRP3 ekspresyonunu (sırasıyla p=0,0008; p=0,0006) anlamlı düzeyde azaltmış; bu da IL-1β salınımında (sırasıyla p=0,0190, p<0,0001), hücre proliferasyonunda (sırasıyla p=0,024; p<0,0001) ve migrasyonunda (sırasıyla p=0,0021; p=0,0004) azalmaya yol açmıştır. Bu bulgular, glukoz metabolizması ve enflamasyonu hedef alarak OSCC tedavisinde Emp'in potansiyel bir terapötik ajan olabileceğini düşündürmektedir.

Sonuç: Bulgularımız, emp'nin SLC2A3 ve NLRP3 ifadesini ve IL-1β salınımını inhibe ederek OSCC'de glikoz metabolizması ve enflamasyonu etkin bir şekilde modüle ettiğini, böylece kanser hücre proliferasyonu ve migrasyonunu azalttığını ve OSCC ilerlemesinin kontrolü için potansiyel bir terapötik ajan olabileceğini göstermektedir.

Anahtar Kelimeler: Oral squamöz hücreli karsinom, SLC2A3, NLRP3, empagliflozin

INTRODUCTION

Elevated glucose metabolism and inflammatory processes are two key factors that facilitate tumor growth, metastasis, and resistance to therapy in oral squamous cell carcinoma (OSCC)[1]. Cancer cells must adapt to rapid microenvironmental changes, such as hypoxia, nutrient scarcity, and acidic pH, particularly when oxidative phosphorylation alone cannot sustain energy production. To compensate, they reprogram glucose metabolism to ensure sufficient adenosine triphosphate (ATP) production and meet biosynthetic demands essential for cell proliferation[2,3]. This metabolic shift enables cancer cells to generate ATP and biosynthetic precursors via aerobic glycolysis, leading to significantly increased glucose uptake in highly proliferative tumor cells[3,4]. This adaptation supplies the substrates necessary for cancer cell proliferation and contributes to tumor progression, metastasis, and long-term survival[4,5].

Additionally, elevated glucose levels in cancer cells can induce reactive oxygen species, which consequently drive the formation of cytoplasmic multiprotein complexes such as the nucleotide-binding oligomerization domain, leucine-rich repeat-containing family pyrin domain containing 3 (NLRP3)[6]. This protein complex, known as the inflammasome, activates inflammatory responses by promoting cytokine secretion, such as pro-inflammatory interleukin-1beta (IL-1β), and releasing it into the extracellular space, thereby intensifying cellular inflammation[7].

Metabolic reprogramming of cancer cells is primarily regulated by hypoxia-inducible factor-1α , which controls the expression of metabolic enzymes, including glucose transporters (GLUTs)[8,9]. Given this metabolic reliance, targeting glucose metabolism offers a promising approach to enhance therapeutic efficacy[10,11]. Among the GLUT family, GLUT3 plays a crucial role in maintaining basal glucose transport, particularly in cells requiring high glucose affinity for survival[12]. In OSCC tumors, GLUT3 expression, encoded by the solute carrier family 2 member 3 *(SLC2A3)* gene, has been linked to poor prognosis and an aggressive cancer phenotype due to enhanced glucose metabolism[13,14]. Moreover, increased NLRP3 expression in OSCC has been associated with tumor progression and metastasis[15].

Given these findings, antidiabetic drugs have been investigated for their potential to modulate cancer cell metabolism and inflammatory responses. While studies have highlighted the anticancer effects of anti-diabetic drugs, including metformin, the role of sodium-glucose cotransporter-2 inhibitors, such as empagliflozin (emp), in cancer treatment remains poorly understood[16-19]. Although emp has been reported to suppress NLRP3 in obesity models[20], its impact on NLRP3 expression remains largely unknown in cancer. Thus, this study examined the influence of emp on SLC2A3, which encodes GLUT3, and NLRP3 in OSCC cell lines. Additionally, we explored the role of Emp-mediated SLC2A3-NLRP3 modulation in OSCC cell proliferation.

MATERIALS AND METHODS

Cells and Reagents

The human recurrent oral cavity squamous cell carcinoma cell line, SCCL-MT1, was generously provided by Bursa University, while the UPSI-SCC-131 OSCC cell line was a gift from (Bursa University). Cells were cultured in Dulbecco's Modified Eagle Medium-high glucose medium supplemented with fetal bovine serum (10%), antibiotic solution (penicillin & streptomycin, 50 U/mL), L-glutamine (2 mM), and sodium pyruvate (1 mM) and maintained under standard culture conditions (37 °C, 5% CO_2, humidified atmosphere). All experiments were conducted using passage 15 cells.

The cell culture media was from HyClone (Logan, UT, USA), while the supplements were provided by BIOCHROME (Berlin, Germany). Lipopolysaccharide (LPS) from Escherichia coli O111:B4 was purchased from Sigma (St. Louis, MO, USA), ATP was purchased from InvivoGen (San Diego, CA, USA) emp was supplied by Boehringer Ingelheim (Biberach, Germany).

Real-Time Polymerase Chain Reaction

Total RNA was isolated by the Quick-RNA Miniprep Kit (Zymo Research, Orange, CA, USA). The concentration and purity of RNA were evaluated by analyzing the A260/280 and A260/230 ratios with a Beckman Coulter DU 730 spectrophotometer (Brea, CA, USA). The ProtoScript M-MuLV First Strand cDNA Synthesis Kit (New England Biolabs, Ipswich, MA, USA) was

used for reverse transcription. The expression level of SLC2A3 was analyzed using the TaqMan™ Gene Expression Assay (Thermo Fisher Scientific, USA), while NLRP3 expression was analyzed with primers reported by Tezcan et al.[21]. Actin-beta (ACTB) served as the reference gene for normalization. For SLC2A3, the quantitative polymerase chain reaction (qPCR) reaction was performed according to the manufacturer's instructions using TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, CA, USA). For NLRP3 and ACTB, qPCR was carried out in a 10 µL reaction volume containing 200 ng of cDNA, 10 µM of each primer, and Luna® Universal qPCR Master Mix (New England Biolabs, Ipswich, MA, USA). qPCR was performed on a StepOnePlus™ real time-PCR system (Thermo Fisher Scientific, Waltham, MA, USA) with the following cycling conditions: initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of denaturation at 95 °C for 10 seconds and annealing/extension at 56 °C for 60 seconds. Data analysis, including Ct value determination and fold change calculations, was conducted using the StepOnePlus™ software and the $2^{-\Delta}$Ct method. For each comparison group, experiments were performed with three independent biological replicates, each analyzed in technical duplicate.

Enzyme-Linked Immunosorbent Assay (ELISA)

A sandwich ELISA approach (SunRed Bio, Shanghai, China) was employed, utilizing a biotin-labeled detection antibody specific to the target IL-1β. Samples and standards were incubated in wells pre-coated with a capture antibody for 1 hour at 37 °C, followed by three washes with phosphate-buffered saline (PBS). The biotin-labeled detection antibody was added and incubated for 30 minutes, followed by the streptavidin-horseradish peroxidase conjugate. After a 30-minute incubation with the substrate solution in the dark, color development was measured at 450 nm (reference: 650 nm) using a microplate reader. Each comparison group was analyzed in three biological repeats, each analyzed in technical duplicates. The results were calculated based on the standard curve generated from IL-1β reference standards.

Dynamic Cell Proliferation Analysis

SCCL-MT1 and UPSI-SCC-131 cells (5×10^4 per well) were plated into an E-Plate 16 (ACEA Biosciences, San Diego, CA, USA). Cell growth was perpetually monitored every 30 minutes over a 96-hour period using the xCELLigence real-time cell analysis (RTCA) dual purpose system (ACEA Biosciences, San Diego, CA, USA), which measures electrical impedance as an indicator of cell viability and proliferation. Background impedance was recorded before cell seeding to ensure accurate normalization. For each of the two different cell lines, the experiment was conducted with three independent biological replicates per comparison group. The resulting proliferation curves were generated and analyzed using the RTCA Software (ACEA Biosciences).

In-vitro Wound Healing Scratch Assay

A scratched wound-healing assay was conducted to visualize cell migration. Once the monolayer achieved confluence, a scratch was introduced using a 1000 µL pipette tip, creating a standardized wound area. Detached cells and debris were carefully removed by washing with PBS. Images of the wounded region were recorded after scratching and again 24 hours post-treatment using an inverted microscope (Nikon, Tokyo, Japan). Wound closure was quantified by measuring the wound area at both time points using ImageJ v1.54f software (National Institutes of Health, Bethesda, MD, USA). The experiment was performed in three biological replicates per comparison group, with each replicate analyzed in technical duplicate to ensure reproducibility.

Statistical Analysis

Protein-protein interactions were evaluated through the STRING v12.0, (https://string-db.org/) (accessed on February 24, 2025). Statistical analysis was achieved by GraphPad Prism (v14.4.0) (GraphPad Software, Boston, MA, USA). Data were confirmed to follow a normal distribution based on Q-Q plot analysis and Shapiro-Wilk tests, with $p>0.05$ indicating no significant deviation from normality. Homogeneity of variances was evaluated using Levene's test. Group differences were analyzed using one-way ANOVA, followed by Tukey's post-hoc test for pairwise comparisons to control for Type I error inflation due to multiple testing. Results are reported as mean ± standard error, with significance defined as $p<0.05$. All statistical tests were conducted with at least three independent biological replicates to ensure data robustness and reproducibility.

RESULTS

Empagliflozin Suppressed SLC2A3 Expression in OSCC Cells

To establish an inflammation-induced OSCC cell model, SCCL-MT1 and UPSI-SCC131 cells were exposed to 1 µg/mL LPS for 3 hours[22]. Additionally, cells were treated with 500 nM Emp, an SGLT2 inhibitor, for 24 hours to suppress glucose transport as described in our previous study[19]. Our results demonstrated that emp significantly downregulated SLC2A3 RNA expression in both SCCL-MT1 ($p<0.0001$), and UPSI-SCC131 cells ($p=0.0002$). Moreover, SLC2A3 expression was further reduced in LPS-induced cells when subsequently exposed to Emp, compared to cells treated with LPS alone ($p<0.0001$) (Figure 1 A, B).

Empagliflozin Reduced NLRP3 Expression and Suppresses IL-1β Release in LPS-Induced Inflammation

Genetic interaction analysis predicted that both SLC2A3 and NLRP3 interact with IL-1β (Figure 2A). Supporting these findings, emp reduced NLRP3 expression in SCCL-MT1 (p=0.0008) and UPSI-SCC131 cells (p=0.0006) (Figure 2B, C). Similarly, in LPS-treated SCCL-MT1 and UPSI-SCC131 cells, the emp treatment led to a decrease in NLRP3 expression than the cells triggered by LPS-only (p<0.0001). To evaluate the effect of emp on IL-1β release upon inflammasome activation, cells were treated with 1 µg/mL LPS for 3 hours, followed by 24-hour emp treatment and 25-minute exposure to 5 mM ATP (Figure 2D). IL-1β release was significantly increased in inflammasome-activated cells compared to those cells under LPS-induced inflammation conditions (p<0.0001). However, emp decreased IL-1β release both in LPS-treated cells (SCCL-MT1: p=0.0190; UPSI-SCC131) (p<0.0001) and in inflammasome-activated cells with LPS-ATP treatment (p<0.0001) (Figure 2E, F), suggesting that Emp, through the inhibition of SLC2A3, may also reduce NLRP3-mediated inflammatory cytokine production.

Empagliflozin Slowed Cell Proliferation and Migration in OSCC Cells

Emp treatment decreased the proliferation of both SCCL-MT1 and UPSI-SCC131 cell lines (Figure 3A, B). In contrast, LPS treatment increased cell proliferation compared to untreated controls. However, emp treatment also inhibited proliferation in LPS-pretreated cells relative to those treated with LPS alone (Figure 3A, B).

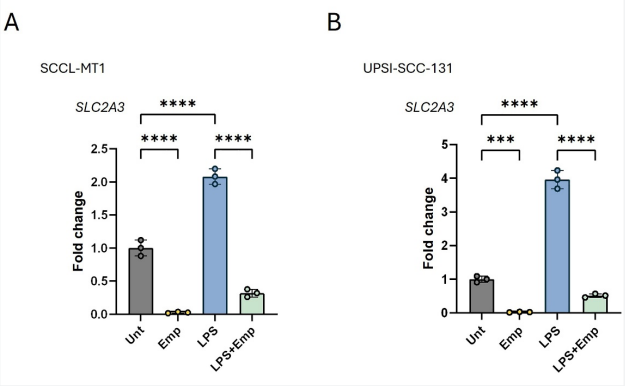

Figure 1. Effect of Emp on SLC2A3 expression in OSCC cells. (A) SCCL-MT1 cells, (B) UPSI-SCC131 cells. Comparison was performed between the unt & emp and LPS & emp+LPS. Only statistically significant differences are shown ***p<0.001, ****p<0.0001

Unt: Untreated, LPS: Lipopolysaccharide, emp: Empagliflozin, OSCC: Oral squamous cell carcinoma

Wound healing assays further supported these findings, showing that emp decreased cell migration in both SCCL-MT1 (p=0.024) and UPSI-SCC131 cells (p<0.0001) (Figure 3C, D). In contrast; LPS enhanced migration, accelerating cell movement (Figure 3C, D). Notably, emp also reduced wound healing in

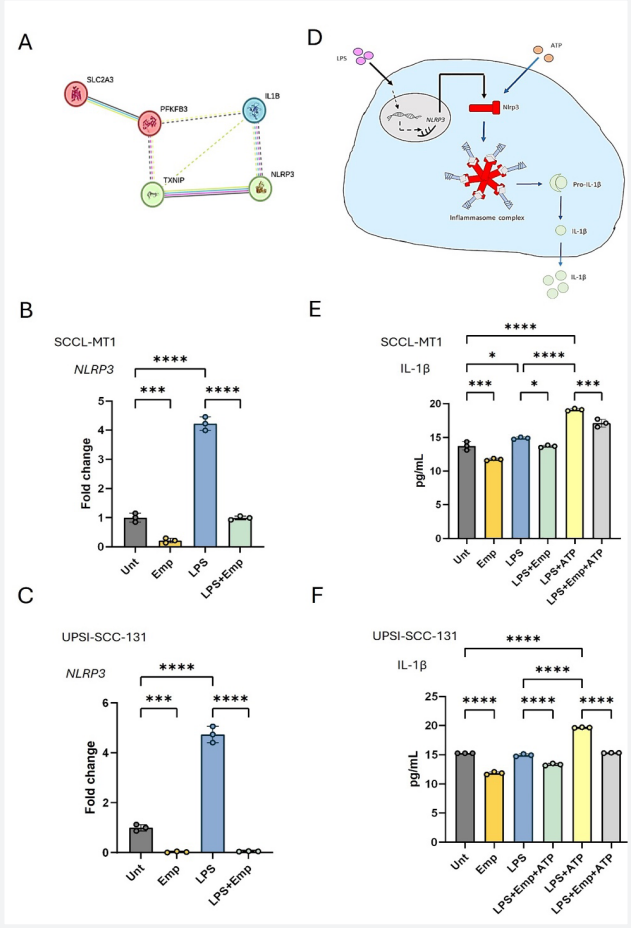

Figure 2. Effect of emp on the NLRP3 inflammasome in OSCC cells. (A) Interaction between SLC2A3, NLRP3, and IL-1β. (B) NLRP3 expression in SCCL-MT1 cells and (C) in UPSI-SCC131 cells. (D) Schematic representation of how LPS and ATP exposure influence NLRP3 transcription and inflammasome complex formation in vitro. (E) IL-1β release from SCCL-MT1 cells and (F) from UPSI-SCC131 cells. Emp, LPS, and LPS+ATP groups were compared to the Unt (control) group. To evaluate the effect of emp under inflammatory conditions, emp+LPS and emp+LPS+ATP groups were compared to LPS. For RT-qPCR analysis, only the comparison between emp+LPS and LPS was performed. Only statistically significant differences are shown.*p<0.05, ***p<0.001, ****p<0.0001

Unt: Untreated, LPS: Lipopolysaccharide, ATP: Adenosine triphosphate, emp: Empagliflozin, OSCC: Oral squamous cell carcinoma, IL-1β: Interleukin-1beta, RT-qPCR: Reverse transcription quantitative polymerase chain reaction

LPS-pretreated cells (SCCL-MT1: p=0.0021; UPSI-SCC131: p=0.0004) (Figure 3C, D). These results suggest that Emp-mediated suppression of SLC2A3 and NLRP3 reduces both the growth rate and invasive potential of OSCC cells, leading to a decrease in aggressive cancer cell behavior.

DISCUSSION

This study investigates the role of emp on NLRP3 inflammasome activation in SCCL-MT1 and UPSI-SCC131, OSCC cell lines. SCCL-MT1, derived from buccal mucosa, is an invasive and recurrent OSCC model with strong immunomodulatory activity[23]. Previous studies have demonstrated that SCCL-MT1 promotes immunosuppression within the tumor microenvironment by stimulating myeloid-derived suppressor cells via IL-1β secretion, leading to effector T-cell suppression and an increase in regulatory T-cells[23,24]. Similarly, UPSI-SCC131, originating from a patient with a history of tobacco and alcohol use, is resistant to radiotherapy[25,26]. This resistance has been associated with elevated IL-1β secretion[27]. Due to their clinically relevant characteristics such as invasiveness, immunomodulatory activity, and therapy resistance, the effects of emp on OSCC were investigated using the SCCL-MT1 and UPSI-SCC131 cell lines in this study.

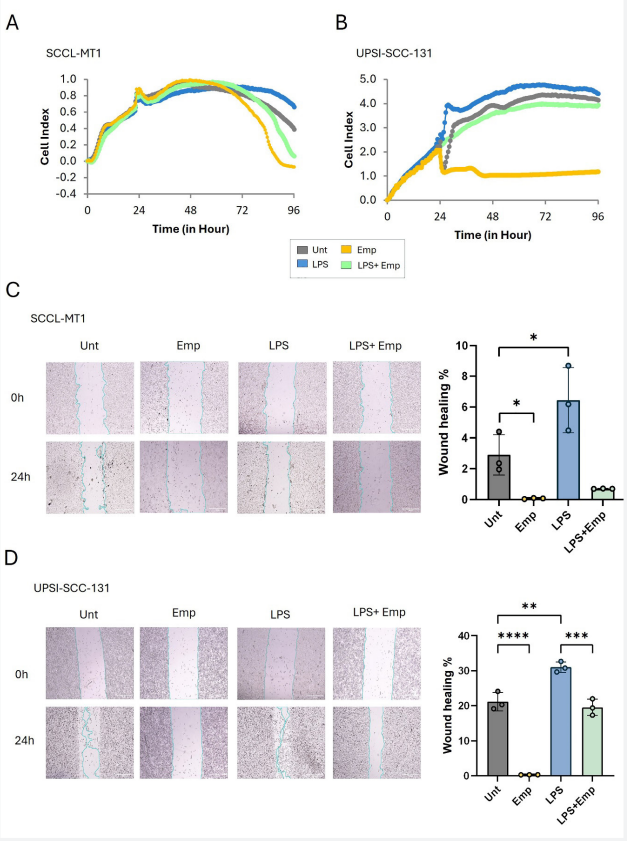

Figure 3. Effect of emp on OSCC cell motility. (A) Real-time proliferation of SCCL-MT1 cells and (B) UPSI-SCC131 cells. (C) Healing of scratched wound in SCCL-MT1 cells and (D) UPSI-SCC131 cells. Comparisons were performed between the Unt & emp and the Unt & LPS groups. In addition, to assess emp's effect under inflammatory conditions, emp+LPS was compared to LPS. Only statistically significant differences are shown. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Unt: Untreated, LPS: Lipopolysaccharide, Emp: Empagliflozin, OSCC: Oral squamous cell carcinoma

Empagliflozin-Mediated Mechanistic Link Between SLC2A3 and NLRP3

IL-1β production and secretion *in vitro* require a two-step activation of the NLRP3 inflammasome. In the priming phase, pathogen-associated stimuli such as LPS activate Toll-like receptors, leading to NF-κB signaling and enhanced NLRP3 transcription. In the activation phase, ATP stimulates the P2X7 receptor, inducing potassium efflux and promoting NLRP3 inflammasome assembly. This process culminates in pro-caspase-1 activation and IL-1β secretion[28-30]. Therefore, we evaluated the effect of emp on NLRP3 activation by stimulating SCCL-MT1 and UPSI-SCC131 cells with LPS, followed by ATP exposure, and analyzing IL-1β secretion.

Building on these mechanistic insights, our findings demonstrate that treatment with Emp, apharmacological inhibitor of glucose transport[31], downregulates SLC2A3 (GLUT3) mRNA expression, indicating reduced glucose uptake in OSCC cells. Emp also suppresses NLRP3 expression and inhibits inflammasome activation, leading to decreased IL-1β secretion. These results suggest a functional link between SLC2A3 and NLRP3 in the regulation of glucose metabolism and inflammation in cancer cells.

A recent study has associated elevated SLC2A3 expression with the activation of epithelial-mesenchymal transition and NF-κB signaling pathways, contributing to poor prognosis in patients with head and neck squamous cell carcinoma[32]. NF-κB plays a pivotal role in initiating inflammasome activation by upregulating the transcription of NLRP3 and pro-IL-1β during the priming phase[33]. In our study, LPS was used to model pathogen-associated molecular patterns. Importantly, even in the absence of LPS stimulation, emp suppressed SLC2A3 and NLRP3 expression, along with IL-1β secretion. These findings suggest that SLC2A3-mediated glucose uptake alone may contribute to inflammasome activation. Indeed, previous studies have shown that elevated glucose transport triggers inflammatory signaling in macrophages[34], the cell type where NLRP3 activation is most pronounced[35].

To further explore this connection, we conducted a gene–gene interaction analysis, which suggested a potential mechanistic link between SLC2A3 and NLRP3 through the glycolysis-regulating enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3). Cancer cells overexpress GLUTs such as SLC2A3 to increase glucose influx, which elevates intracellular fructose-2,6-bisphosphate (F2,6BP) levels. This metabolite allosterically activates phosphofructokinase-1, the rate-limiting enzyme in glycolysis. PFKFB3 amplifies this effect by promoting F2,6BP synthesis, accelerating glycolytic flux and enhancing lactate production. Lactate is exported from the cell, bypassing mitochondrial oxidative metabolism and promoting reliance on aerobic glycolysis. This shift supports rapid proliferation and fosters a hypoxic tumor microenvironment[36]. Emerging evidence indicates that PFKFB3 not only promotes glycolytic flux but also contributes to NLRP3 inflammasome activation. Suppression of PFKFB3 has been shown to reduce glycolysis[36], and in macrophages, NLRP3 can regulate glycolysis through PFKFB3[37]. Moreover, PFKFB3-induced hypoxia has been reported to trigger NLRP3 activation, while downregulation of PFKFB3 reduced NLRP3 downstream targets, including caspase-1 and IL-1β, in an atherosclerosis model[38]. Notably, LPS has been shown to enhance ROS generation induced by high glucose–associated hypoxia, thereby exacerbating NLRP3 activation[39].

Taken together, these findings support the hypothesis that the SLC2A3-PFKFB3-NLRP3 axis may represent a potential mechanistic link between altered glucose metabolism and inflammation in OSCC (Figure 4). Although based on in silico predictions, this pathway suggests that by suppressing SLC2A3, emp may attenuate NLRP3 inflammasome activation, potentially through modulation of PFKFB3, thereby exerting both metabolic and anti-inflammatory effects in oral cancer.

Therapeutic Implications of Emp on OSCC

The role of the NLRP3 inflammasome in cancer remains controversial. While some studies associate NLRP3 overexpression with tumor aggressiveness and poor prognosis, others report minimal effects upon its inhibition[40]. Our previous work using Glibenclamide and VX765, two NLRP3 inhibitors, showed only modest reductions in OSCC cell proliferation[22,41]. In contrast, emp exhibited a more pronounced anti-cancer effect, significantly decreasing OSCC cell proliferation and impairing wound healing, suggesting greater therapeutic potential through dual metabolic and inflammatory modulation.

Notably, SLC2A3 is known to promote OSCC progression through the TGF-β signaling[42]. In addition, TGF-β signaling has been implicated in chronic inflammation through an IL-1β-driven autocrine mechanism[43]. Although not tested in this

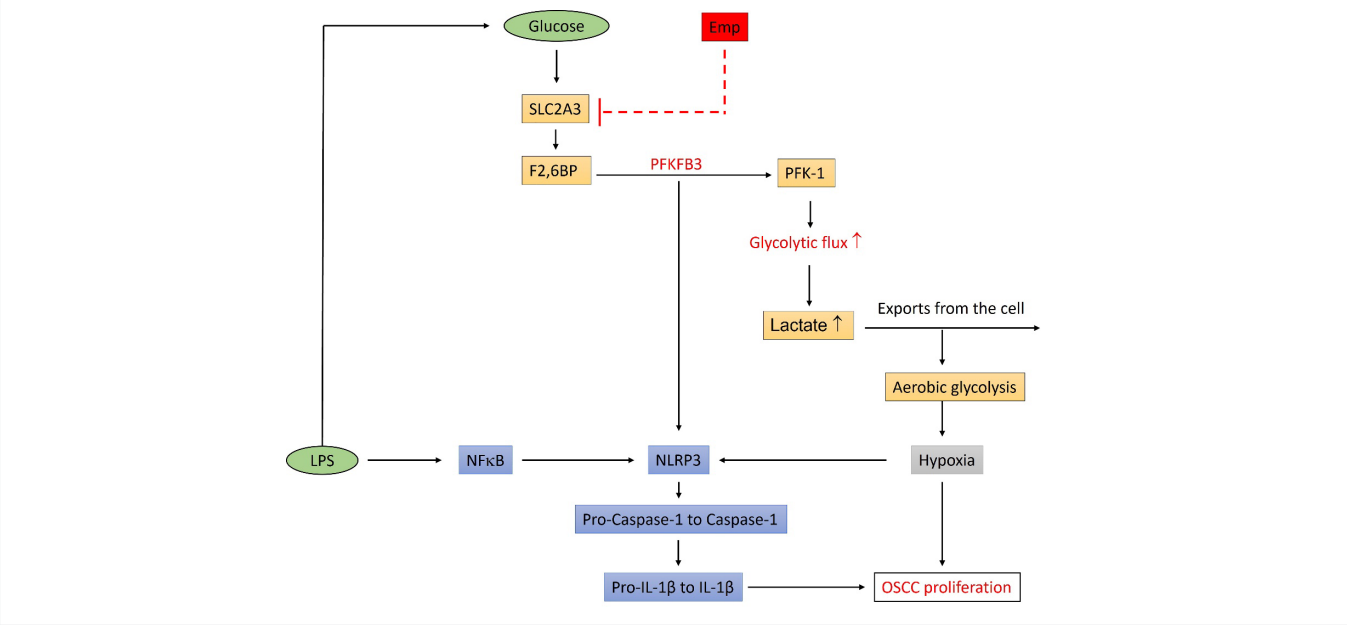

Figure 4. Schematic representation of the hypothetical SLC2A3-PFKFB3-NLRP3 Axis and emp's action in OSCC. Emp suppresses glucose uptake by downregulating SLC2A3, which may lead to decreased PFKFB3 activity and subsequently reduce NLRP3 inflammasome activation. This proposed pathway links altered glucose metabolism to inflammation in OSCC

LPS: Lipopolysaccharide, emp: Empagliflozin, OSCC: Oral squamous cell carcinoma, LPS: Lipopolysaccharide, SLC2A3: Solute carrier family 2 member 3

study, emp's reported inhibition of TGF-β signaling[44] suggests a potential additional mechanism for its effects in OSCC, which warrants further investigation. The convergence of SLC2A3-mediated glucose transport and TGF-β–IL-1β signaling pathways may represent a novel therapeutic target axis in OSCC.

Collectively, these data support the hypothesis that glucose transport contributes to OSCC aggressiveness, and its inhibition by emp may help suppress this phenotype. Under LPS-induced inflammatory conditions, proliferation and migration rates increased but were effectively reduced by Emp. These findings highlight emp's potential not only as an antidiabetic agent but also as a repurposed therapeutic candidate targeting metabolic-inflammation crosstalk in OSCC.

Comparison of Emp and Conventional NLRP3 Inhibitors

Mechanistically, Glibenclamide prevents inflammasome formation by blocking NLRP3 monomer assembly, while VX765 inhibits caspase-1 activation, which is required for IL-1β maturation[22,41,45]. Although emp does not directly target the inflammasome complex, our results suggest it reduces NLRP3 expression by limiting glucose availability, a key upstream stimulus. Thus, emp may act earlier in the NLRP3 pathway and exert additional effects via SLC2A3-associated signaling.

Overall, this study reveals that metabolic regulation of glucose uptake plays a critical role in the progression of inflammation associated with OSCC cells. Notably, the indirect interaction between SLC2A3 and NLRP3 may contribute to shaping this inflammatory response. In this context, the suppression of proliferation and migration observed after treatment with glucose transport inhibitors such as emp likely results from the downregulation of NLRP3 expression. This reduction may limit NLRP3-associated signaling pathways involved in inflammation, thereby decreasing the secretion of pro-tumorigenic cytokines.

Indeed, the effects of emp under both basal and LPS-induced inflammatory conditions highlight the potential of glucose uptake inhibition to reduce pro-inflammatory signaling and disrupt autocrine cytokine loops in cancer cells, thereby mitigating the aggressive phenotype of OSCC.

Study Limitations

This study is an *in vitro* investigation conducted using two commercial cell lines. In OSCC inflammation, the inflammatory activities of non-cancerous cells within the tumor microenvironment and their interactions with cancer cells play a role. However, the experimental setup in this study consists of a homogeneous system containing only cancer cells, thereby excluding the influence of the tumor microenvironment. This represents a limitation of our study.

Furthermore, the study employed a single concentration of Emp, selected based on preliminary cytotoxicity and efficacy data. Although effective, the use of a single dose restricts dose–response interpretation and does not account for possible concentration-dependent effects or therapeutic windows. Additional experiments using multiple concentrations and time points would enhance the pharmacodynamic understanding of emp in OSCC.

Another important limitation is the lack of *in vivo* validation. Without animal model data, it is difficult to predict the systemic effects of Emp, its pharmacokinetics, or its potential interactions within a living organism.

Moreover, as this is not a patient-based study, the focus is solely on the effects of emp on cancer cell biology, disregarding potential variations arising from patient heterogeneity, etiological differences, and epigenetic diversity. These factors may limit the clinical generalizability and translational applicability of our findings.

CONCLUSION

In conclusion, our findings demonstrate that Emp, an SGLT2 inhibitor, suppresses SLC2A3 and NLRP3 expression, thereby reducing OSCC cell proliferation and migration. Its dual action, disrupting glucose metabolism and mitigating chronic inflammation via NLRP3 inhibition, may attenuate the aggressive phenotype of OSCC. Taken together, these insights highlight emp's potential as a therapeutic agent for OSCC, though further studies are needed to validate its clinical efficacy.

Ethics

Ethics Committee Approval: The study does not involve human or animal subjects, ethical approval is not required. The relevant statement has also been included in the manuscript.

Informed Consent: This study is an in vitro study conducted using two commercially available cell lines. No biological material derived from patients or experimental animals was used, and no clinical or personal data from any patient were included.

Footnotes

Authorship Contributions

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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