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BRIEF REPORT

Transcriptional regulation of glucose transporters in human oral squamous cell carcinoma cells

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Abstract

The increased glucose uptake observed in cancer cells is mediated by glucose transporters (GLUTs), a class of transmembrane proteins that facilitate the transport of glucose and other substrates across the plasma membrane. Despite the important role of glucose in the pathophysiology of oral squamous cell carcinoma (OSCC), there is very limited data regarding the expression of GLUTs in normal or malignant cells from the oral mucosa. We analysed the messenger RNA (mRNA) expression of all 14 GLUTs in two OSCC (H357/H400) and one non-malignant oral keratinocyte (OKF6) cell line using a quantitative polymerase chain reaction. GLUT expression was evaluated at baseline and after treatment with two specific GLUT inhibitors, namely, BAY876 (GLUT1) and WZB117 (GLUT1, GLUT3 and GLUT4). Here, we show that GLUT1, GLUT3, GLUT4, GLUT5, GLUT6, GLUT8, GLUT12 and GLUT13 transcripts were measurably expressed in all cell lines while GLUT2, GLUT7, GLUT9, GLUT11 and GLUT14 were not expressed. GLUT10 was only found in H357. In the presence of BAY876 and WZB117, OSCC cells exhibited significant alterations in the transcriptional profile of GLUTs. In particular, we observed distinct proliferation-dependent changes of mRNAs to GLUT1, GLUT3, GLUT4, GLUT5 and GLUT6 in response to selective GLUT inhibitors. In summary, we documented for the first time the expression of GLUT5, GLUT6 and GLUT12 in normal and malignant oral keratinocytes. Whilst regulation of GLUT transcripts was cell line and inhibitor specific, GLUT3 was consistently upregulated in actively proliferating OSCC cell lines, but not in OKF6, regardless of the inhibitor used, suggesting that modulation of this transporter may act as one of the primary compensation mechanisms for OSCC cells upon inhibition of glucose uptake.

KEYWORDS

glucose transporter, GLUT, oral cancer, oral squamous cell carcinoma, SGLT

Rita Paolini and Caroline Moore are co-first authors.

1 | INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the ninth most prevalent cancer globally, with 354 864 new cases diagnosed and 177 384 deaths in 2018.[1] Despite advancements in treatment, the 5-year overall survival of approximately 50% has remained unchanged in the past few decades.[2] Dysregulation of metabolic pathways is a key hallmark of cancer and OSCCs rely upon aerobic glycolysis as their primary method of ATP production.[3] The glucose transporter (GLUT) family is part of the major facilitator superfamily and consists of 14 transmembrane proteins coded by SLC2A genes. GLUTs are primarily known for the transport of glucose and other hexose molecules, such as fructose,[4,5] and their distribution can vary widely across tissues. GLUT expression has previously been correlated with poor prognosis, chemoresistance and radioresistance in several cancers including OSCC.[6,7]

We have recently shown that OSCC pathophysiology and resistance to treatment in part rely on glucose utilization by cancer cells.[8] Furthermore, our recent systematic review on GLUTs in OSCC revealed that GLUT1, GLUT3 and GLUT4 are the most well studied and commonly expressed GLUTs in OSCC and that these transporters are also frequently overexpressed in malignant oral epithelial cells compared to normal oral keratinocytes (NOK).[9] However, insufficient data was available for the other members of the GLUT family to draw meaningful conclusions regarding their role in OSCC.

In the present study, we investigated the expression of GLUTs in cells from both normal oral mucosa and OSCC as well as their response to selective GLUT inhibitors.

2 | METHODS

The messenger RNA (mRNA) expression of GLUTs was analysed in two OSCC (H357 and H400) and one NOK (OKF6) cell line using quantitative polymerase chain reaction (qPCR). The OSCC adherent cell lines H357/H400 were established from primary explants of OSCC from the tongue and alveolar process, respectively.[8] All 14 GLUT mRNAs were assessed as well as GAPDH mRNA as an internal control. To assess if cell growth would affect the response to the inhibitors compared to cells that exit the cell cycle after contact inhibition, cells were grown to two different cell densities (50% confluent or actively proliferating and 100% confluent or quiescent)[10] and GLUT expression evaluated at baseline and after GLUT inhibitor treatments. Cells were treated with 500 nM BAY876 (GLUT1 inhibitor; A17216; ADOOQ Bioscience) and 25 μM WZB117 (GLUT1, GLUT3 and GLUT4 inhibitor; 19900; Cayman Chemical Company) 24 h before they were expected to reach the chosen confluency. At the endpoint, the six-well plates were kept on ice throughout the cell-lysis process. Four hundred microlitres of cold lysis buffer (Buffer RTL- RNeasy Mini Kit [Qiagen Australia]) was added to each well. Cells were scraped from the surface of the wells using a cell scraper (541070; Greiner). All three technical replicates for each experimental condition were pooled, and 600 μl of lysate was collected for RNA extraction. RNA extraction was completed using the RNeasy Kit (74004; Qiagen). The concentration of total RNA samples was determined on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and RNA sample integrity was assessed on a TapeStation 4200 (Agilent Technologies). Total RNA extracted were all shown to be of high-quality preparations, both containing absorbance ratios of 260/280 nm of 2 and RNA Integrity Number values of 9 or 10. The QuantiTect Reverse Transcription Kit (Qiagen Australia) was used to generate cDNA from a 300 ng total RNA template as per the manufacturer's instructions. qPCR analysis was performed with QuantiTect SYBR Green PCR Master Mix (Qiagen Australia) and 10X QuantiTect Primer Assays (Qiagen Australia) for each of the GLUTand GAPDH qPCR primers (Table 1). The cycling conditions for the AriaMX Thermocycler were: 95°C for 15 min, 40 cycles of 94°C for 15 s, 53°C for 30 s and 72°C for 30 s. The $2^{-\Delta\Delta Ct}$ method was used to determine the fold changes between samples. Fold changes were compared using a two-tailed, paired *t*-test with a significance value of $p < 0.05$.

TABLE 1 QuantiTect Primer Assays used in GLUT mRNA qPCR analysis (Qiagen Australia).

Gene	Symbol	GeneGlobe ID #	CAT #
GLUT1	Hs_SLC2A1_1_SG	QT00068957	249900
GLUT2	Hs_SLC2A2_2_SG	QT01008399	249900
GLUT3	Hs_SLC2A3_1_SG	QT00047124	249900
GLUT4	Hs_SLC2A4_1_SG	QT00097902	249900
GLUT5	Hs_SLC2A5_1_SG	QT00087766	249900
GLUT6	Hs_SLC2A6_1_SG	QT00040866	249900
GLUT7	Hs_SLC2A7_2_SG	QT01677116	249900
GLUT8	Hs_SLC2A8_1_SG	QT00036729	249900
GLUT9	Hs_SLC2A9_1_SG	QT00079695	249900
GLUT10	Hs_SLC2A10_1_SG	QT00049602	249900
GLUT11	Hs_SLC2A11_1_SG	QT00035574	249900
GLUT12	Hs_SLC2A12_1_SG	QT00078113	249900
GLUT13	Hs_SLC2A13_1_SG	QT00098315	249900
GLUT14	Hs_SLC2A14_1_SG	QT00044520	249900
GAPDH	Hs_GAPDH_1_SG	QT00079247	249900

Abbreviations: GLUT, glucose transporter; mRNA, messenger RNA; qPCR, quantitative polymerase chain reaction.

3 | RESULTS AND DISCUSSION

This is the first study assessing all 14 GLUTs in a normal keratinocyte cell line (OKF6), and two OSCC cell lines (H357 and H400). We detected basal expression of mRNA for GLUT1, GLUT3, GLUT4, GLUT5, GLUT6, GLUT8, GLUT12 and GLUT13 in all cell lines whereas GLUT10 transcripts were only found in H357. GLUT2, GLUT7, GLUT9, GLUT11 and GLUT14 were not detected at the mRNA level in the cell lines tested. This is also the first time that expression of GLUT5, GLUT6 and GLUT12 has been reported in oral keratinocytes. Furthermore, the expression of GLUT7 and GLUT14 had never been

assessed in OSCC and we have shown that the mRNA levels of these two GLUTs are undetectable in OSCC cells in vitro.

3.1 | Expression of GLUT mRNAs in actively growing and quiescent oral keratinocyte monolayers

The mRNA levels of GLUTs that were expressed in all cell lines and at different seeding densities are summarised in Figures 1 and 2. Expression of GLUT1 and GLUT13 was consistently higher in OKF6 compared to OSCC cells, whereas mRNA levels of other GLUTs varied (Figure 1). Except for GLUT5, actively dividing non-malignant oral keratinocytes (OKF6) seem to express higher or similar levels of GLUT transcripts compared to 100% confluent cells. Conversely, GLUT mRNA levels were often lower in actively dividing (50% confluent) OSCC cells compared to 100% confluent cells (Figure 2). Specifically, there was an increase in nearly all GLUT mRNA levels in fully confluent H357 cells except GLUT6 which was downregulated, whereas GLUT4, GLUT8 and GLUT10 levels remained stable. In H400 cells, GLUT expression was not significantly affected by proliferation, except for GLUT6 and GLUT13 which showed reduced and increased expression at 100% confluency, respectively. At confluency, H400 also displayed increased expression in GLUT1, GLUT4, GLUT5 and GLUT12, but these changes were not statistically significant. GLUT13 and GLUT6 were the only GLUTs which were significantly over- and under-expressed at 100% confluency in both OSCC cell lines.

3.2 | Changes in GLUT expression pattern upon selective GLUT inhibition

We subsequently investigated whether the expression of GLUTs was modified by selective GLUT inhibition. Figure 3 summarises the levels of mRNA expression compared to each cell's baseline without an inhibitor present. In general, OKF6 were less responsive to GLUT inhibitors compared to cancer cells. In one example, specific inhibition of GLUT1 in OKF6 by BAY876 did not affect the levels of any GLUT transcripts except GLUT6, which however was upregulated only in actively dividing cells (Figure 3). Similarly, simultaneous inhibition of GLUT1, GLUT3 and GLUT4 by WZB117 was associated with changes

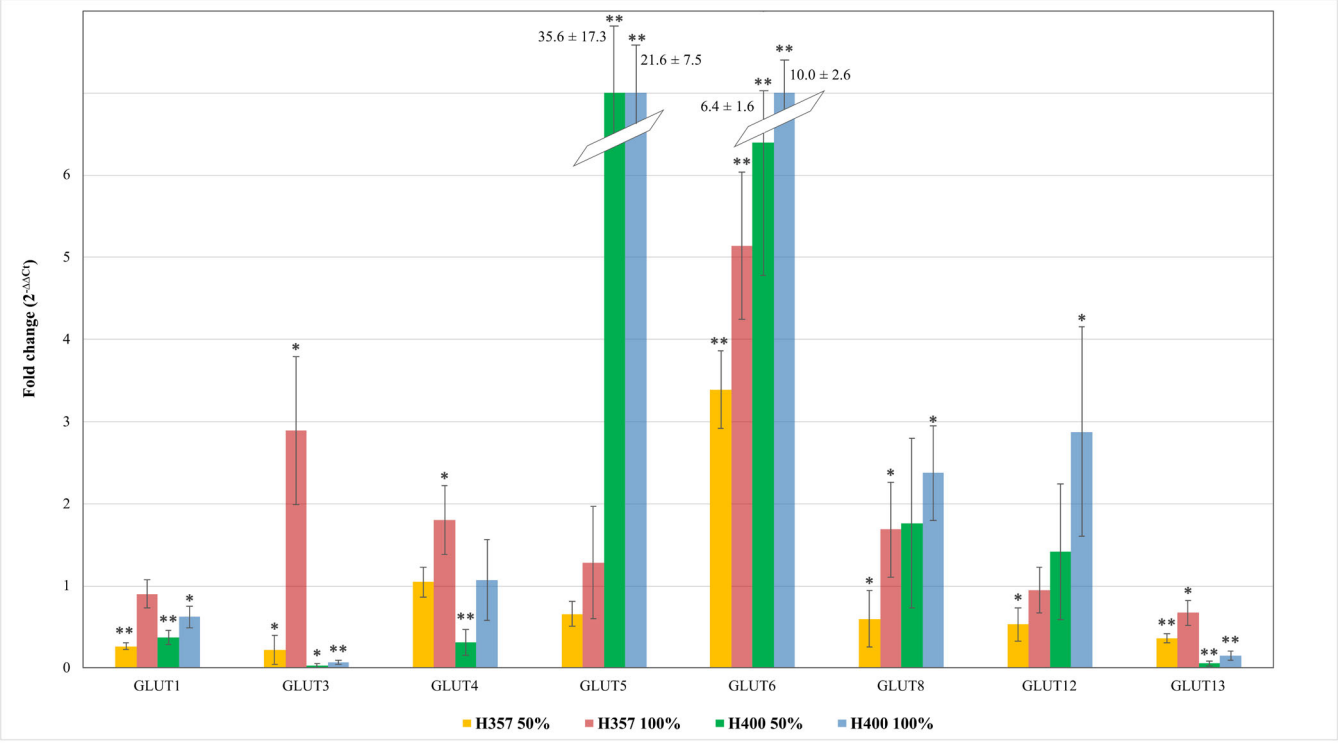

FIGURE 1 Fold change compared to NOK of mRNA GLUT expression in oral squamous cell carcinomas at 50% and 100% confluence. Each data point was obtained from three biological replicates and error bars represent the SD. For the control, the SD is an averaged SD from all groups.

in the mRNA expression levels of GLUT4, GLUT5, GLUT6 and GLUT12. Interestingly, GLUT6 transcription displayed a divergent response to BAY876 versus WZB117 in proliferating normal oral keratinocytes, suggesting GLUT1 and GLUT3 and GLUT4 have opposite roles in the regulation of this transporter in normal oral keratinocytes. In OSCC cells, transcriptional regulation of GLUTs by GLUT inhibition was more profound. Proliferating H400 cells responded to GLUT1 inhibition with increased transcription of GLUT1, whereas the opposite was true in quiescent cells. A similar trend was observed in H357 cells, albeit not statistically significant. The same proliferation-dependent regulation by selective GLUT inhibitors was observed for various transporters. Specifically, GLUT4 (H400) and GLUT6 (H357) were selectively upregulated in proliferating but not in quiescent cancer cells in the presence of BAY876, while GLUT3 displayed this biphasic behaviour in both OSCC cell lines. Conversely, GLUT5 mRNA levels were diminished by BAY876 treatment in OSCC cells.

When GLUT1 was inhibited simultaneously along with GLUT3 and GLUT4 (WZB117), the mRNA expression pattern was modified significantly. For example, the proliferation-specific effects on OSCC cells were abolished (GLUT1 and GLUT6, the latter only for H357) or reverted (GLUT4 downregulation). Interestingly, the changes observed for GLUT3 and GLUT5 expression patterns were similar for BAY876 and WZB117 in both OSCC cell lines.

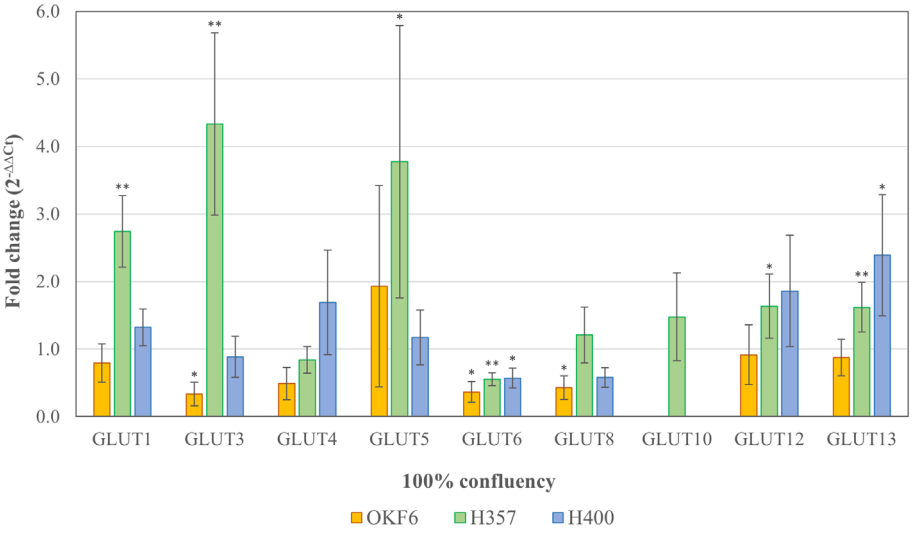

FIGURE 2 Fold change difference between 50% and 100% confluence of mRNA GLUT expression. Each data point was obtained from three biological replicates and error bars represent the SD. For the control, the SD is an averaged SD from all groups.

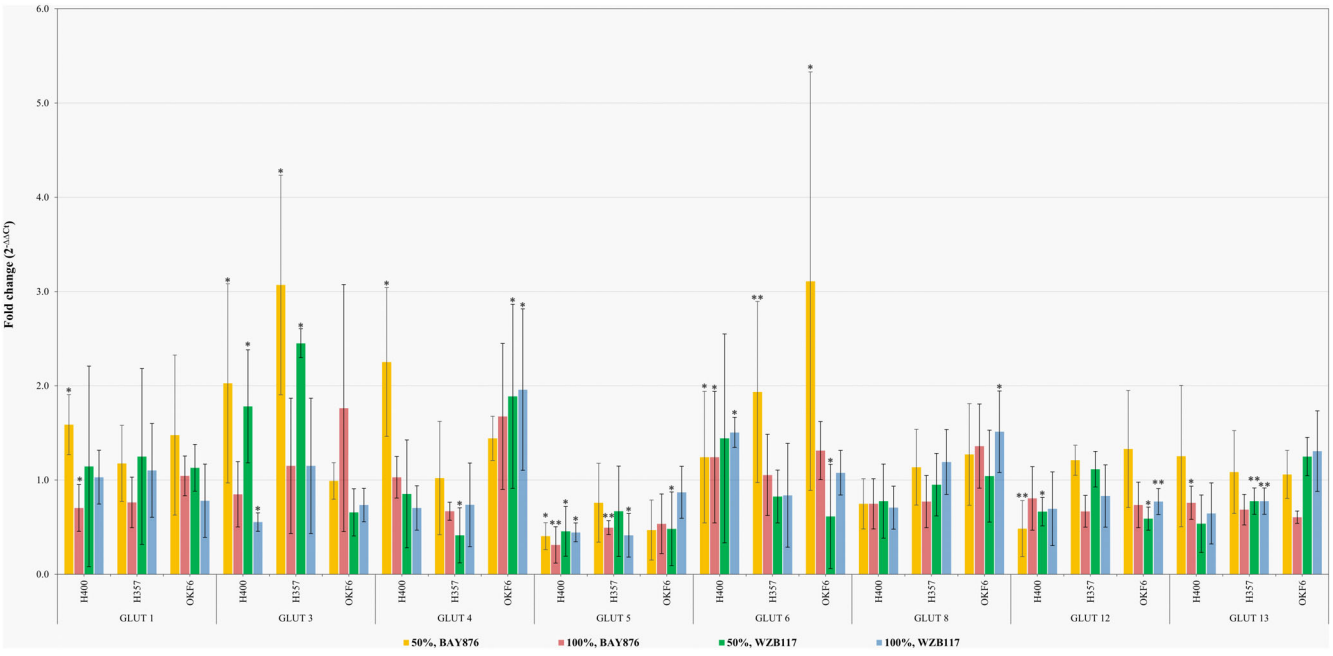

FIGURE 3 Fold change difference between 50% and 100% confluence of GLUT mRNA expression in OKF6 and oral squamous cell carcinoma cells when treated with BAY876 or WZB117. Each data point was obtained from 3 biological replicates and error bars represent the SD. The control was baseline expression with no inhibitor present and the SD is an averaged SD from all groups.

In summary, GLUT mRNA expression was affected by treatment with BAY876 and WZB117 and this was dependent on the confluency of the cells. With few exceptions, actively proliferating OSCC cells expressed lower basal levels of GLUTs and were more susceptible to transcriptional regulation by GLUT inhibitors. It is possible that the observed changes in the GLUT transcriptional profile may occur to compensate for the inhibited GLUT. Interestingly, some of the GLUTs not directly targeted by the inhibitors were found to be downregulated. It may be that the inhibitors had a global effect on transcription due to their impact on glucose, and therefore ATP production. Finally, our experiments were designed to detect mRNA expression, hence we are unable to comment as to whether the observed changes in mRNA correspond to GLUT protein expression. Proteomic GLUT analysis in OSCC is therefore an area for future focus.

4 | CONCLUSION

In the present study, we undertook a comprehensive assessment of GLUTs in normal and malignant oral keratinocytes. For the first time, we report the expression of GLUT5, GLUT6 and GLUT12 in oral keratinocytes. Our results also show that pharmacological inhibition of GLUT1, GLUT3 and GLUT4 induced transcriptional modifications in most GLUTs, which was particularly marked in actively proliferating OSCC cells. A key feature that emerged from our study was the upregulation of GLUT3 in proliferating OSCC cells, but not in non-malignant cells, following inhibition of GLUT1 or GLUT1, GLUT3, GLUT4. Hence, upregulation of GLUT3 appears to be a consistent compensation mechanism in oral cancer cells in response to glucose deprivation and could therefore represent a potential therapeutic target for OSCC.

AUTHOR CONTRIBUTIONS

Conception and design: Rita Paolini and Antonio Celentano. *Development of methodology*: Rita Paolini, Caroline Moore and Antonio Celentano. *Acquisition of data*: Rita Paolini, Caroline Moore and Antonio Celentano. *Analysis and interpretation of data*: Rita Paolini, Caroline Moore, Tamara Matthyssen, Nicola Cirillo, Tami Yap and Antonio Celentano. *Writing, review and/or revision of the manuscript*: Rita Paolini, Caroline Moore, Tamara Matthyssen, Nicola Cirillo, Michael McCullough, Camile Farah, Heinrich Botha, Tami Yap and Antonio Celentano. *Study supervision*: Rita Paolini and Antonio Celentano.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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