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

Rita Paolini | Caroline Moore | Tamara Matthyssen | Nicola Cirillo | Michael McCullough | Camile S. Farah | Heinrich Botha | Tami Yap | Antonio Celentano

1Melbourne Dental School, The University of Melbourne, Carlton, Victoria, Australia
2Australian Centre for Oral Oncology Research & Education, Nedlands, Western Australia, Australia
3Oral, Maxillofacial and Dental Surgery, Fiona Stanley Hospital, Murdoch, Western Australia, Australia
4Head and Neck Pathology, Australian Clinical Labs, Subiaco, Western Australia, Australia

Correspondence
Antonio Celentano, Melbourne Dental School, The University of Melbourne, 720 Swanston Street, Carlton, VIC 3053, Australia.
Email: antonio.celentano@unimelb.edu.au

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, 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, GLUT6 and GLUT12 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
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. Despite advancements in treatment, the 5-year overall survival of approximately 50% has remained unchanged in the past few decades. Dysregulation of metabolic pathways is a key hallmark of cancer and OSCCs rely upon aerobic glycolysis as their primary method of ATP production. 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, 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.

We have recently shown that OSCC pathophysiology and resistance to treatment in part rely on glucose utilization by cancer cells. 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). 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. All three technical replicates for each experimental condition were pooled, and 600 μl of lysisate 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 GLUT and GAPDH qPCR primers (Table 1).

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

| Gene   | Symbol     | GeneGlobe ID # | CAT # |
|--------|------------|----------------|-------|
| GLUT1  | Hs_SLC2A1_1_SG | QTO0068957    | 249900|
| GLUT2  | Hs_SLC2A2_2_SG | QTO1008399    | 249900|
| GLUT3  | Hs_SLC2A3_1_SG | QTO0047124    | 249900|
| GLUT4  | Hs_SLC2A4_1_SG | QTO0097902    | 249900|
| GLUT5  | Hs_SLC2A5_1_SG | QTO0087766    | 249900|
| GLUT6  | Hs_SLC2A6_1_SG | QTO0040866    | 249900|
| GLUT7  | Hs_SLC2A7_2_SG | QTO1677116    | 249900|
| GLUT8  | Hs_SLC2A8_1_SG | QTO0036729    | 249900|
| GLUT9  | Hs_SLC2A9_1_SG | QTO0079695    | 249900|
| GLUT10 | Hs_SLC2A10_1_SG | QTO0049602   | 249900|
| GLUT11 | Hs_SLC2A11_1_SG | QTO0035574    | 249900|
| GLUT12 | Hs_SLC2A12_1_SG | QTO0078113    | 249900|
| GLUT13 | Hs_SLC2A13_1_SG | QTO0098315    | 249900|
| GLUT14 | Hs_SLC2A14_1_SG | QTO0044520    | 249900|
| GAPDH  | Hs_GAPDH_1_SG | QTO0079247    | 249900|

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

The cycling conditions for the AriaMX Thermocycler were: 95°C for 15 s, 53°C for 30 s and 72°C for 30 s. The ΔΔCt 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.

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.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the Melbourne Dental School, The University of Melbourne. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jop.13342.

DATA AVAILABILITY STATEMENT

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

ORCID

Rita Paolini https://orcid.org/0000-0002-2170-4723
Caroline Moore https://orcid.org/0000-0002-1152-9802
Tamara Matthyssen https://orcid.org/0000-0003-0053-7679
Nicola Cirillo https://orcid.org/0000-0003-1429-1323
Michael McCullough https://orcid.org/0000-0001-7829-4684
Camile S. Farah https://orcid.org/0000-0002-1642-6204
Heinrich Botha https://orcid.org/0000-0001-6075-4603
Tami Yap https://orcid.org/0000-0001-8412-1114
Antonio Celentano https://orcid.org/0000-0003-4293-2511

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
2. Marsh D, Suchak K, Moutasim KA, et al. Stromal features are predictive of disease mortality in oral cancer patients. J Pathol. 2011;223(4):470-481.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646-674.
4. Hediger MA, Clemençon B, Burrier RE, Bruford EA. The ABCs of membrane transporters in health and disease (SLC series): introduction. Mol Aspects Med. 2013:34:95-107.
5. Burant C, Takeda J, Brot-Laroche E, Bell G, Davidson N. Fructose transporter in human spermatozoa and small intestine is GLUT5. J Biol Chem. 1992;267:14523-14526.
6. Kunkel M, Moergel M, Stockinger M, et al. Overexpression of GLUT-1 is associated with resistance to radiotherapy and adverse prognosis in squamous cell carcinoma of the oral cavity. Oral Oncol. 2007;43(8):796-803.
7. Bhatt AN, Chauhan A, Khanna S, et al. Transient elevation of glycolysis confers radio-resistance by facilitating DNA repair in cells. BMC Cancer. 2015;15:335.
8. Celentano A, McCullough M, Cirillo N. Glucocorticoids reduce chemotherapeutic effectiveness on OSCC cells via glucose-dependent mechanisms. J Cell Physiol. 2019;234(3):2013-2020.
9. Botha H, Farah CS, Koo K, et al. The role of glucose transporters in oral squamous cell carcinoma. Biomolecules. 2021;11(8):1070.
10. Gos M, Miloszewska J, Swoboda P, Trembacz H, Skierski J, Janik P. Cellular quiescence induced by contact inhibition or serum withdrawal in C3H10T1/2 cells. Cell Prolif. 2005;38(2):107-116.

How to cite this article: Paolini R, Moore C, Matthyssen T, et al. Transcriptional regulation of glucose transporters in human oral squamous cell carcinoma cells. J Oral Pathol Med. 2022;51(8):679-683. doi:10.1111/jop.13342