European Review for Medical and Pharmacological Sciences 2018; 22: 7726-7734

Inhibition of microRNA-218 promotes oral squamous cell carcinoma growth by targeting GLUT1 to affect glucose metabolism

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Abstract. – OBJECTIVE: Glucose transporter 1 (GLUT1) is a key player in glucose metabolism that has important roles in oral squamous cell carcinoma (OSCC), while microRNA-218 can target GLUT1 to achieve its biological roles. Therefore, we hypothesize that microRNA-218 may target GLUT1 to participate in OSCC.

PATIENTS AND METHODS: Tumor tissues and adjacent healthy tissues were collected from OSCC patients, and blood samples were collected from both OSCC patients and healthy controls. Expression of microRNA-218 and GLUT1 in those tissues was detected by qRT-PCR. All patients were followed up for 5 years. Diagnostic and prognostic values of serum microRNA-218 for OSCC were investigated by ROC curve analysis and survival curve analysis, respectively. MicroRNA-218 knockdown OSCC cell lines were established. The effects on cell proliferation, glucose uptake as well as GLUT1 expression were detected by CCK-8 assay, glucose uptake assay and Western blot.

RESULTS: MicroRNA-218 expression level was decreased while GLUT1 expression level was increased in tumor tissues compared with adjacent healthy tissues. Serum level of microRNA-218 was lower, while serum level of GLUT1 was higher in cancer patients than that in healthy control. Serum microRNA-218 and GLUT1 can be used to accurately predict OSCC and its prognosis. MicroRNA-218 knockdown promoted tumor cell proliferation, increased glucose uptake and promoted GLUT1 expression.

CONCLUSIONS: Inhibition of microRNA-218 can promote oral cancer cell growth by targeting GLUT1 to affect glucose metabolism.

Key Words:
Oral squamous cell carcinoma, Glucose transporter 1, microRNA-218.

Introduction

About 16-40% of all malignancies are head and neck cancers[1]. As a major type of malignancy in head and neck, oral squamous cell carcinoma (OSCC) represents about 95% of all forms of head and neck cancers[2]. Smoking and excessive alcohol consumption have been proved to be major risk factors for OSCC[3]. However, increased incidence of this disease in developed world among a younger population showed less association with excessive alcohol or tobacco consumption[4], indicating the complex pathogenesis of the disease. In spite of the improvements in radiotherapy, chemotherapy and targeted therapy, surgery is still the preferable treatment for patients with OSCC. However, prognosis of this disease after proper surgical operation is relatively limited due to local invasion and metastasis, which is a leading cause of postoperative recurrence[1]. Therefore, the development of novel treatment approaches and identification of novel targets for the treatment is always needed. Glucose metabolism is important for the growth of normal cells as well as tumor cells, and inhibition of glucose metabolism has been proved to inhibit the growth of certain types of cancers[5]. As a major player in glucose metabolism, glucose transporter 1, or GLUT1, also has an important function in the development of various types of malignant tumors including OSCC[6]. MicroRNA-218 is a tumor-suppressive miRNA, and its expression can inhibit the growth and metastasis of certain types of malignancies[7]. It has been reported[8] that microRNA-218 can target GLUT1 to achieve its biological functions. However, interactions between microRNA-218 and GLUT1 in

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OSCC still have not been well investigated. Therefore, we aimed to investigate the interactions between microRNA-218 and GLUT1 in OSCC.

Patients and Methods

Patients

From January 2010 to January 2012, a total of 68 patients with OSCC enrolled in the Third Affiliated Hospital of Sun Yat-sen University were selected to serve as research subjects. All those patients were confirmed with OSCC by pathological and imaging examinations. Those 68 patients included 28 males and 30 females, and the age ranged from 24 to 72 years, with an average age of 47±11.4 years. Staging of primary tumors was performed according to the following standards: T1, the greatest dimension $\leq$ 2 cm; T2, 2 cm < greatest dimension <4 cm; T3, greatest dimension $\geq$ 4 cm; T4, tumor invades through cortical bone, inferior alveolar nerve, into deep/extrinsic muscle of tongue, floor of mouth, masticator space, pterygoid plates, skin, or skull base, or encases internal carotid artery. There were 14 cases of T1, 14 cases of T2, 16 cases of T3 and 24 cases of T4. All patients received surgical resection and tumor tissues as well as adjacent healthy tissues were collected during surgical operations. At the same time, a total of 28 people with normal physical conditions were enrolled as controls, and control group included 16 males and 12 females. And age ranged from 21 to 70 years, with an average age of 45±12.9. The Ethics Committee of our hospital approved this study, and all patients signed informed consent.

Cell Lines and Cell Culture

Human OSCC cell line SCC09 cell, SCC15 and SCC25 and normal human oral cell line PCS-200-014 were purchased from ATCC. All cell lines were cultured according to the instructions that provided by ATCC. Cells were harvested during logarithmic growth phase for subsequent experiments.

Construction of microRNA-218 Knockdown Cell Lines

MiR-218-specific antisense inhibitor (5'-ACAUGGUUAGAUCAAGCACAA-3') and negative control oligonucleotide (5'-CAGUACUUUUGUGUAGUACAA-3') were provided by GenePharma (Shanghai, China). Cells were cultured over night to reach 80-90% confluent, and then Lipofectamine 2000 reagent (11668-019, Invitrogen, Carlsbad, CA, USA) was used to performed transfection.

Preparation of Serum Samples

Fasting blood (20 ml) was collected from each participant in the morning of the admission day. Blood samples were kept at room temperature for 2 hours, followed by centrifugation at 2000 rpm for 15 min to separate serum. Serum samples were stored at -80°C before RNA extraction.

Cell Proliferation Assay

Cell counting kit 8 (CCK-8, Sigma-Aldrich, St. Louis, MO, USA) was used to detect cell proliferation. Briefly, 100 μl of cell suspension containing $4x10^4$ cells were added to each well of a 96-well plate. Cells were cultured and CCK-8 solution (10 uL) was added into each well 24, 48, 72 and 96 h later. Cells were cultured at 37°C for another 4 h, and OD values at 450 nm were measured with Fisherbrand™ accuSkan™GO UV/Vis Microplate Spectrophotometer (Fisher Scientific, Waltham, MA, USA).

Glucose Uptake Assay

Cells were cultured in 6-well plates with about 5×10^5 cells in each well for 24 h. After that, cells were washed twice with PBS, followed by incubation in 2 ml of Krebs-Ringer-HEPES (KRH) buffer (120 mM NaCl, 25 mM Hepes, pH 7.4, 1.2 mM $MgSO_4$, 5 mM KCl, 1.3 mM $CaCl_2$ 1.3 mM KH_2PO_4) containing 1 μCi of [3H]-2-deoxyglucose (Perkin Elmer Life Sciences, Waltham, MA, USA) at 37°C for 20 min to initiate the experiments. Next, cells were washed twice with ice-cold KRH buffer to halt uptake. Then cells were lysed in 300 μl of lysis buffer (0.2% SDS, 10 mM Tris–HCl, pH 8.0). Liquid scintillation spectrometry was used to measure radioactivity. Intracellular level of [3H]-2-deoxyglucose was represented by disintegrations per minute (DPM), and each assay was performed for three times.

Real-Time Quantitative PCR

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from tumor tissues, adjacent healthy tissues, *in vitro* cultured cells and serum. All RNA samples were tested using NanoDrop™ 2000 Spectrophotometers (Thermo Fisher Scientific, Waltham, MA, USA), and only the ones with an A260/A280 ratio between 1.8 and 2.0 were used in reverse transcription to synthesize cDNA. PCR reaction system was

prepared using SYBR® Green Real-Time PCR Master Mixes (Thermo Fisher Scientific, Waltham, MA, USA). Following primers were used in PCR reactions: 5'- TTGTGCTTGATCTAACCATGT -3' (forward) and Uni-miR qPCR Primer (reverse, Sigma-Aldrich, St. Louis, MO, USA) for microRNA-218; 5'-AACTCTTCAGCCAGGGTCCAC-3' (forward); reverse: 5'-CACAGTGAAGATGATGAAGAC-3' (reverse) for GLUT1; 5'-GACCTCTATGCCAACACAGT-3' (forward) and 5'-AGTACTTGCGCTCAGGAGGA-3' (reverse) for β-actin. PCR reaction conditions were: 95°C for 1 min, followed by 40 cycles of 95°C for 10 s and 60°C for 40 s. Data were processed using $2^{-\Delta\Delta CT}$ method. Relative expression level of each gene was normalized to endogenous control β-actin.

Western-Blot

RIPA solution (Thermo Fisher Scientific, Waltham, MA, USA) was used to extract total protein from *in vitro* cultured cells, and BCA method was used to quantify protein samples. After that, 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed, followed by transmembrane to polyvinylidene difluoride (PVDF) membrane. After blocking with 5% skimmed milk for 2 h at room temperature, membranes were washed and then incubated with primary antibodies including rabbit anti-GLUT1 (1:2000, ab15309, Abcam, Cambridge, MA, USA) and anti-GAPDH primary antibody (1:1000, ab8245, Abcam, Cambridge, MA, USA) overnight at 4°C. The next day, membranes were further incubated with anti-rabbit IgG-HRP secondary antibody (1:1000, MBS435036, MyBioSource) at room temperature for 2 h, followed by addition of ECL (Sigma-Aldrich, St. Louis, MO, USA) to detect the signals. Signals were scanned using MYECL™ Imager (Thermo Fisher Scientific, Waltham, MA, USA), and Image J software was used to normalize relative expression level of TGF-β1 to endogenous control GAPDH.

Statistical Analysis

All data were processed using SPSS19.0 (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA). Count data were expressed as rate and processed by x^2 test. Measurement data were expressed as ($\bar{x}\pm s$), and comparisons between two groups were performed by *t*-test. Comparisons among multiple groups were performed by analysis of variance and LSD test. $p < 0.05$ was considered to be statistically significant.

Results

Expression of microRNA-218 and GLUT1 in Tumor Tissues and Adjacent Healthy Tissues of Patients With Oral Squamous Cell Carcinoma

Expression of microRNA-218 and GLUT1 in tumor tissues and adjacent healthy tissues of 68 patients with OSCC was detected by qRT-PCR. As shown in Figure 1a, expression of microRNA-218 was significantly downregulated in tumor tissues compared with adjacent healthy tissues in 64 out of 68 patients ($p < 0.05$). In contrast, expression of GLUT-1 was significantly upregulated in tumor tissues compared with adjacent healthy tissues in 58 out of 68 patients ($p < 0.05$). Those data suggest that downregulation of miRNA-218 and upregulation of GLUT1 are very likely to be involved in the pathogenesis of OSCC.

Expression of microRNA-218 and GLUT1 in Serum of Healthy Controls and Patients with Different Stages of Oral Squamous Cell Carcinoma

As shown in Figure 2a, expression level of microRNA-218 in serum was significantly higher in serum of healthy controls than that in patients with different stages of OSCC. In addition, expression level of microRNA-218 was further decreased with the increased stage of primary tumor. In contrast, serum level of GLUT-1 was significantly lower in healthy controls than that in patients with different stages of OSCC. In addition, expression level of GLUT-1 was increased with the increased stage of primary tumor.

Diagnostic and Prognostic Value of serum microRNA-218 and GLUT1 for Oral Squamous Cell Carcinoma

ROC curve analysis was performed to evaluate the diagnostic values of serum microRNA-218 and GLUT1 for OSCC. The area under the curve (AUC) of serum microRNA-218 in the diagnosis of OSCC was 0.9498 with 95 interval of 0.9108 to 0.9889 ($p < 0.0001$, Figure 3a). AUC of serum GLUT-1 in the diagnosis of OSCC was 0.8876 with 95 interval of 0.8203 to 0.950 ($p < 0.0001$, Figure 3b). Those data suggest that serum microRNA-218 and GLUT1 may serve as potential biomarkers in the diagnosis of OSCC. Patients were divided into high expression and low expression groups according to the median serum level of microRNA-218 and GLUT1, respecti-

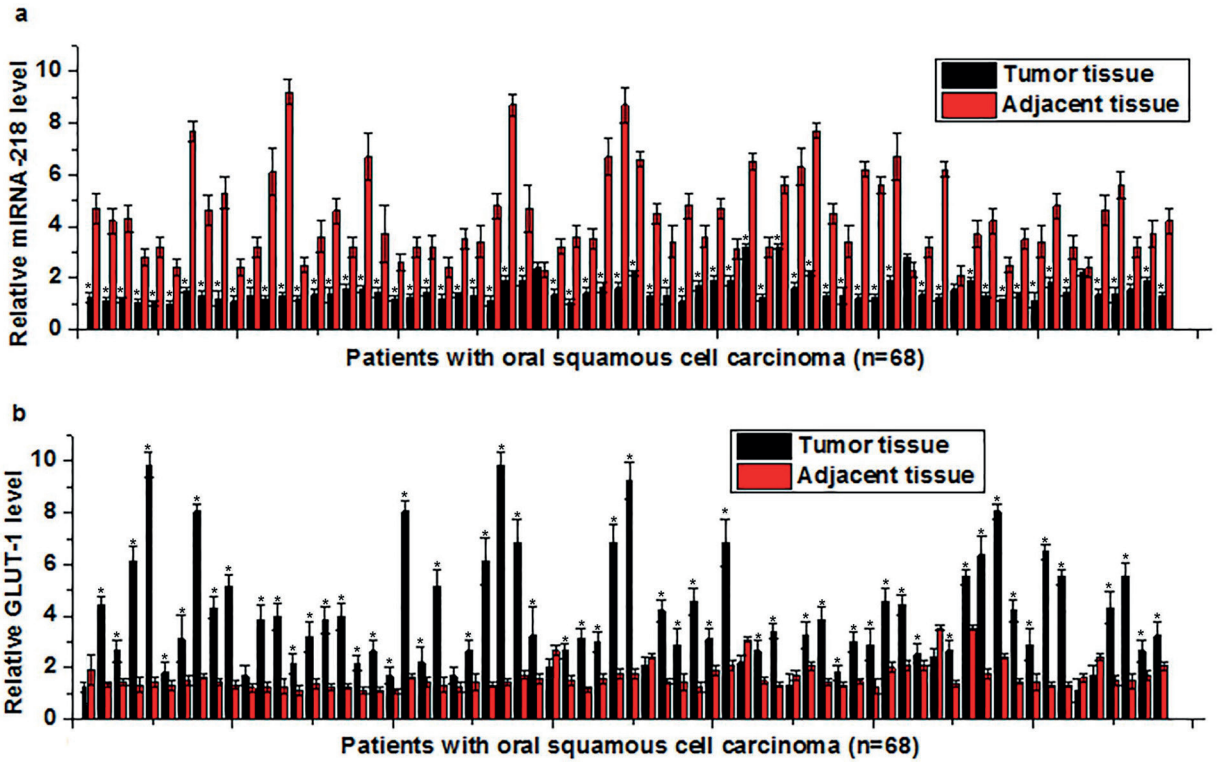

Figure 1. Expression of microRNA-218 and GLUT1 in tumor tissues and adjacent healthy tissues of patients with OSCC. a) Expression of microRNA-218 in tumor tissues and adjacent healthy tissues of patients with OSCC; b) Expression of GLUT1 in tumor tissues and adjacent healthy tissues of patients with OSCC. Notes: *compared with adjacent healthy tissue, $p<0.05$.

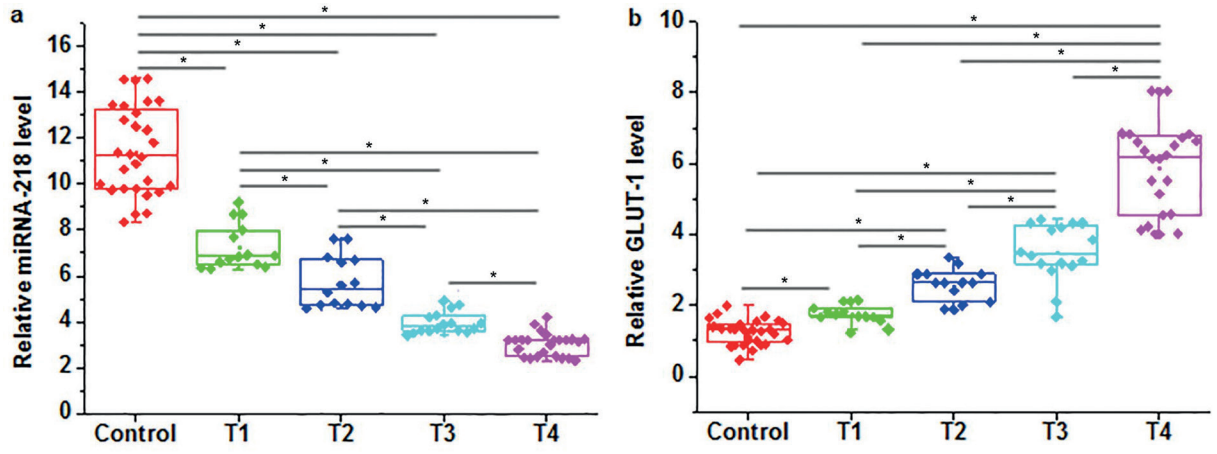

Figure 2. Expression of microRNA-218 and GLUT1 in serum of healthy controls and patients with different stages of OSCC. a) Expression of microRNA-218 in serum of healthy controls and patients with different stages of OSCC; b) Expression of GLUT1 in serum of healthy controls and patients with different stages of OSCC. Notes: *, $p<0.05$

vely. Survival curves were plotted using Kaplan-Meier method and compared by log-rank *t*-test. As shown in Figure 3c, the overall survival of patients with high expression level of microRNA-218 was significantly higher than that of patients with low expression level of microRNA-218. In contrast, overall survival of patients with high expression level of GLUT1 was significantly lower than that of patients

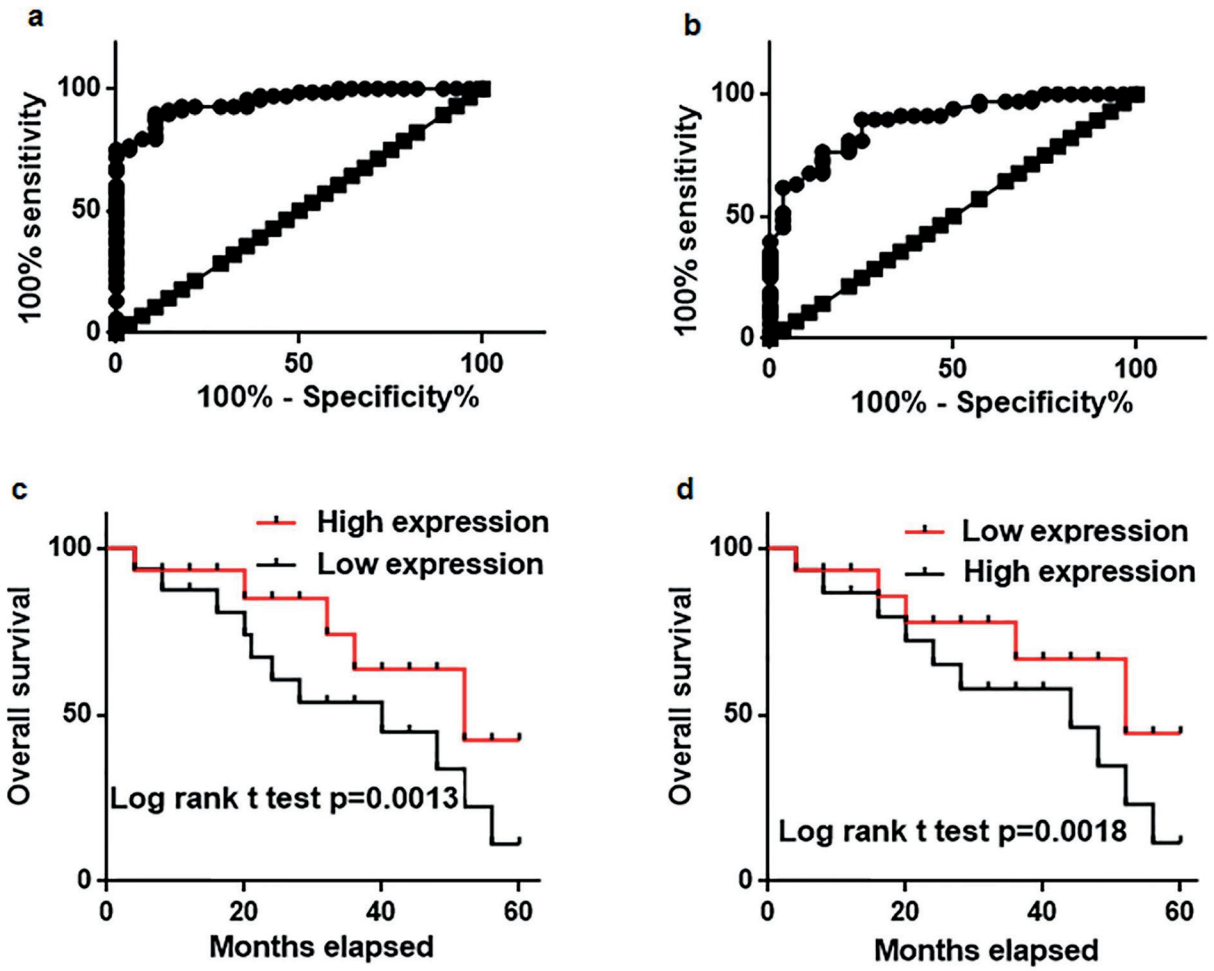

Figure 3. Diagnostic and prognostic value of serum microRNA-218 and GLUT1 for OSCC. a) Diagnostic values of serum microRNA-218 analyzed by ROC curve analysis; b) Diagnostic values of serum GLUT1 analyzed by ROC curve analysis; c) Comparison of survival curves of patients with high and low serum level of microRNA-218; d) Comparison of survival curves of patients with high and low serum level of GLUT1.

with high expression level of GLUT1. Those data suggest that serum microRNA-218 and GLUT1 are two promising diagnostic and prognostic biomarkers for OSCC.

Inhibition of microRNA-218 Expression Promoted the Proliferation of Oral Squamous Cell Carcinoma Cells

As shown in Figure 4a, expression of microRNA-218 was significantly lower in three OSCC cell lines than that in normal oral cell line PCS-200-014. In contrast, significantly higher expression level GLUT-1 was found in OSCC cell lines than in normal oral cell line. After transfection of microRNA-218 inhibitor, expression level of microRNA-218 was significantly reduced compared with control cells (untransfected cells) and cells that transfected with negative control nucleotides (data not shown), which indicated the successfully established microRNA-218 knockdown cell lines. As shown in Figure 4b, compared with control cells and negative control cells, cells with microRNA-218 expression inhibition showed significantly reduced proliferation rate. Those data indicate that microRNA-218 expression inhibition can promote the proliferation of OSCC cells.

MicroRNA-218 Expression Inhibition Promoted GLUT1 Expression and Increased Glucose Uptake

GLUT1 is a key player in glucose metabolism, which plays important roles in OSCC. MicroRNA-218 can play its biological roles by targeting GLUT1. In this study, MicroRNA-218 expression inhibition significantly increased the expression level of GLUT1 in all three OSCC cell lines at

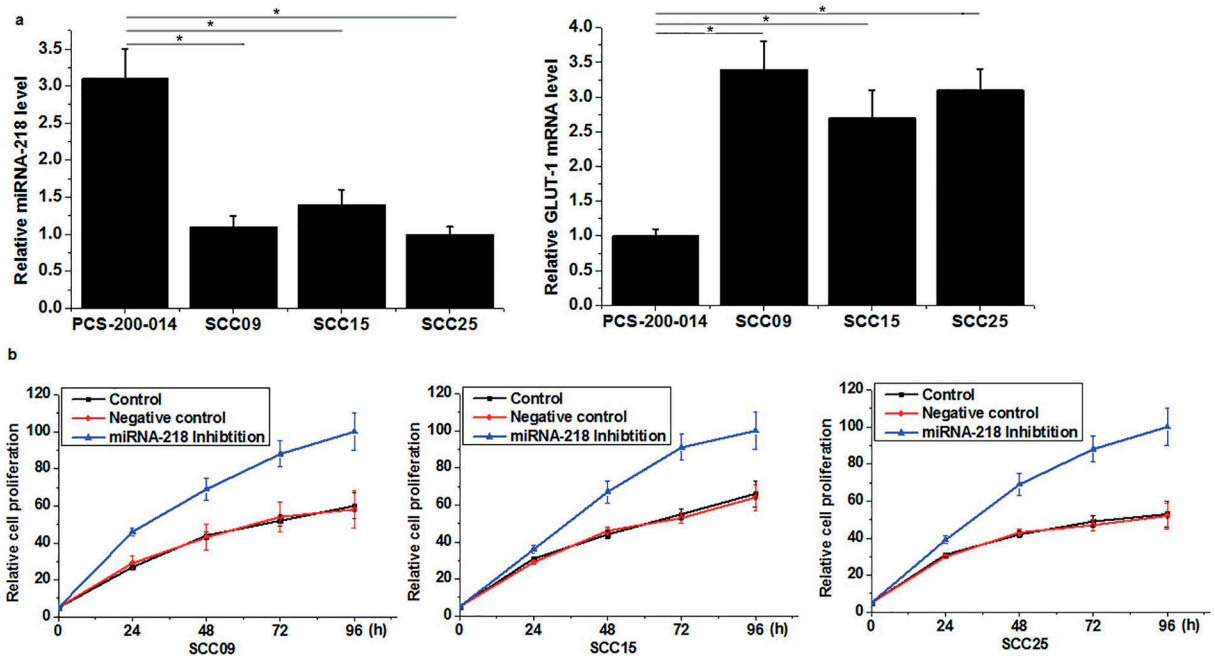

Figure 4. Inhibition of microRNA-218 expression promoted the proliferation of OSCC cells. a) Expression of microRNA-218 in OSCC cell lines and normal oral cell; b) proliferation of three OSCC cell lines with different treatment. Notes: *, $p<0.05$

protein level ($p < 0.05$, Figure 5a). In addition, microRNA-218 expression inhibition also significantly increased the glucose uptake of those three OSCC cell lines ($p < 0.05$, Figure 5b). Those data suggest that inhibition of microRNA-218 promotes OSCC growth by upregulating GLUT1 so as to affect glucose metabolism.

Discussion

Altered metabolic programs are common in cancer cells, and the characteristic changes may include enhanced rates of glutaminolysis and fatty acids synthesis as well as increased uptake of glucose. The accelerated metabolic processes can support the growth and survival of tumor cells[9]. As an important glucose transporter, GLUT1 also showed important roles in the growth and survival of different types of cancer cells[10], therefore the abnormal expression pattern of GLUT1 is usually observed in cancer cells compared with normal healthy cells. In the study of prostate cancer, Gonzalez-Menendez et al[11] found that GLUT1 protein expression level was increased in cancer patients compared with normal controls, and the expression level of GLUT-1 was further increased with the progression of cancer. GLUT1 expression level was also increased in lung cancer, and the increased expression level of GLUT1 was proved to closely correlate with malignant grades[12]. MiRNA-218 is a tumor suppressor miRNA that can play its biological role by inhibiting the proliferation migration, invasion and metastasis of certain types of cancers[13,14]. Expression of miRNA-218 is usually downregulated in the development of certain types of malignancies, such as pancreatic ductal adenocarcinoma[15]. In our study, the expression of GLUT-1 was significantly upregulated, while miRNA-218 was significantly downregulated in tumor tissues than in adjacent healthy tissues for most patients with OSCC. In addition, serum level of GLUT-1 was significantly lower in healthy controls than in patients with different stages of OSCC, and expression level of GLUT-1 was further increased with the increased stage of primary tumor. In contrast, miRNA-218 showed an opposite expression pattern during the progression of OSCC. Those results suggest that downregulation of miRNA-218 and upregulation of GLUT1 are very likely to be involved in the development and progression of OSCC. Clinical application of surgical operation, which is the only radical treatment for OSCC, is very limited due to the distant metastasis. In addition, survi-

val of OSCC is challenged by the poor prognosis, which is mainly caused by postoperative recurrence[1]. Therefore, the accurate early diagnosis and reliable prediction of prognosis will definitely improve the overall survival of those patients. In this study, ROC curve analysis showed that both serum miRNA-218 and GLUT1 could be used to accurately predict OSCC. In addition, comparisons of survival curves showed that high expression level of miRNA-218 and low expression level of GLUT1 are conductive for the survival of those patients. Those data suggest that serum miRNA-218 and GLUT1 may serve as effective and accurate biomarkers for the diagnosis and prognosis of OSCC patients. MiRNA-218 expression has been proved to inhibit the growth of tumors by inhibiting the proliferation of tumor cells in several types of malignancies such as bladder cancer[16], pancreatic cancer[17], and hepatocellular carcinoma[18]. Reduced expression of miRNA-218 promoted cancer cell proliferations[19]. In this study, significantly reduced proli-

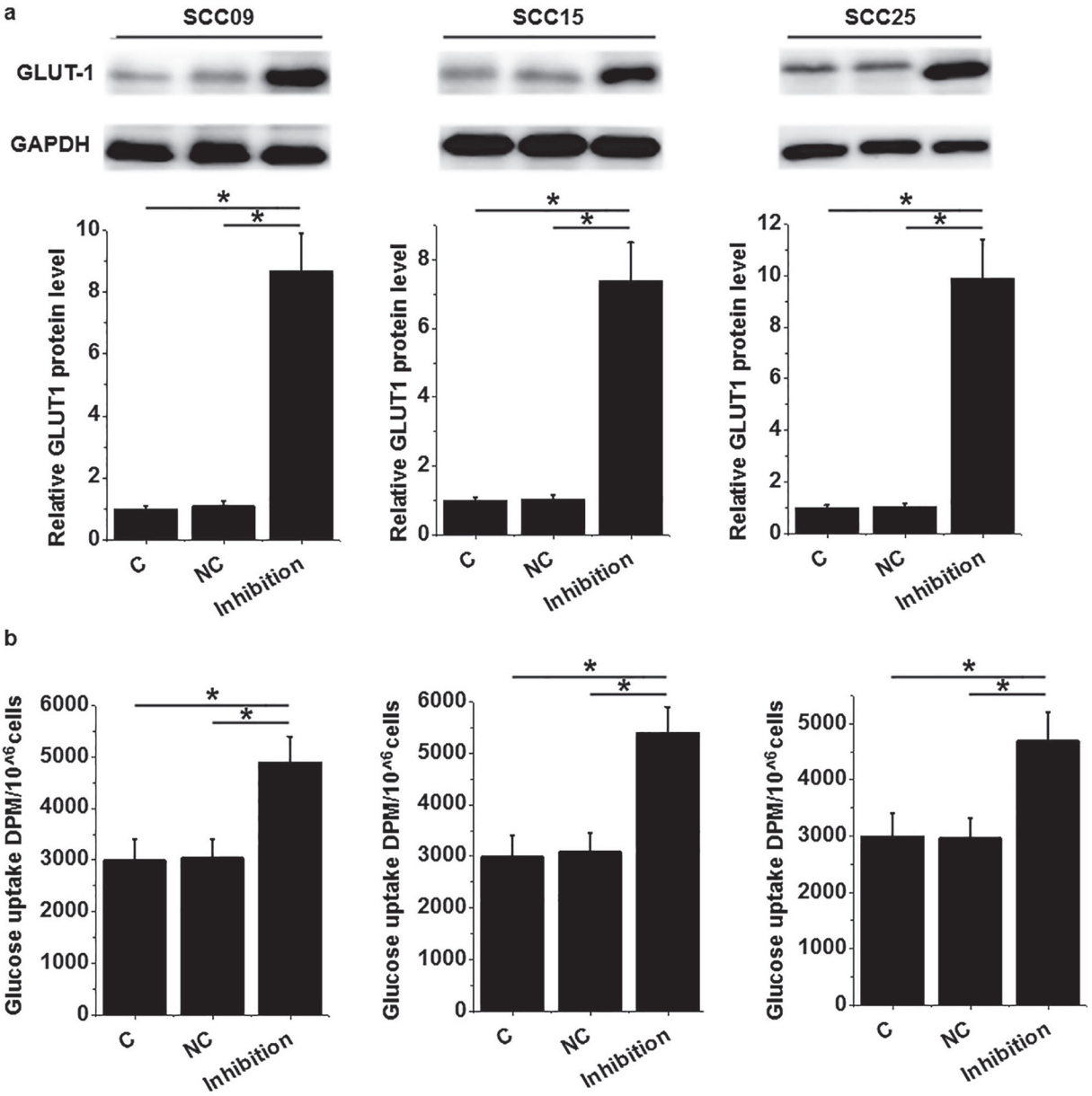

Figure 5. MicroRNA-218 expression inhibition promoted GLUT1 expression and increased glucose uptake. a) Expression of GLUT in three OSCC cell lines; b) Glucose uptake of three OSCC cell lines. Notes: *, $p<0.05$.

feration rates were observed in three OSCC cell lines after the transfection of miRNA-218, indicating that miRNA-218 expression inhibition can promote the growth of OSCC. It has been reported that microRNA-218 can play its biological roles by targeting GLUT1. In this study, MicroRNA-218 expression inhibition significantly increased the expression level of GLUT1 in all three OSCC cell lines at protein level. In addition, microRNA-218 expression inhibition also significantly increased the glucose uptake of those three OSCC cell lines. Those data suggest that Inhibition of microRNA-218 promoted OSCC growth by upregulating GLUT1 to affect glucose metabolism.

Conclusions

We found that microRNA-218 expression level was decreased while GLUT1 expression level of GLUT1 was more increased in tumor tissues than in adjacent healthy tissues. In addition, serum level of microRNA-218 was lower, while serum level of GLUT1 was higher in cancer patients than that in healthy controls. Both serum microRNA-218 and GLUT1 were proved to accurately predict OSCC and its prognosis. MicroRNA-218 knockdown promoted tumor cell proliferation, promoted GLUT1 expression and increased glucose uptake. Therefore, we conclude that inhibition of microRNA-218 can promote oral cancer cell growth by targeting GLUT1 to affect glucose metabolism.

Acknowledgments

We thank the financial support from Technology Project of Guangdong Province (No. 2016A020215203).

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Wang B, Zhang S, Yue K, Wang XD. The recurrence and survival of oral squamous cell carcinoma: a report of 275 cases. Chin J Cancer 2013; 32: 614618.
2) Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma. Oncol Lett 2014; 8: 7-11.
3) Al-Hebshi NN, Nasher AT, Speicher DJ, Shaikh MH, Johnson NW. Possible interaction between tobacco use and EBV in oral squamous cell carcinoma. Oral Oncol 2016; 59: e4-e5.
4) Dalley AJ, Pitty LP, Major AG, Abdulmajeed AA, Farah CS. Expression of ABCG2 and Bmi-1 in oral potentially malignant lesions and oral squamous cell carcinoma. Cancer Med 2014; 3: 273-283.
5) O'Neal J, Clem A, Reynolds L, Dougherty S, Imbert-Fernandez Y, Telang S, Chesney J, Clem BF. Inhibition of 6-phosphofructo-2-kinase (PFKFB3) suppresses glucose metabolism and the growth of HER2+ breast cancer. Breast Cancer Res Treat 2016, 160: 29-40.
6) Xu P, Li Y, Zhang H, Li M, Zhu H. MicroRNA-340 mediates metabolic shift in oral squamous cell carcinoma by targeting glucose transporter-1. J Oral Maxillofac Surg 2016; 74: 844-850.
7) Kumamoto T, Seki N, Mataki H, Kamikawaji K, Takagi K, Suetsugu T, Watanabe M, Mizuno K, Samukawa T, Inoue H. Tumor-suppressive MicroRNA-218 inhibits cancer cell migration and invasion directly targeting TPD52 in lung squamous cell carcinoma. A71. Oncogenes and angiogenesis in lung tumors. American Thoracic Society 2017: A2371.
8) Li P, Yang X, Cheng Y, Zhang X, Yang, C, Deng X, Li P, Tao J, Yang H, Wei J, Tang J, Yuan W, Lu Q, Xu X, Gu M. MicroRNA-218 increases the sensitivity of bladder cancer to cisplatin by targeting Glut1. Cell Physiol Biochem 2017, 41: 921-932.
9) Fadaka A, Ajiboye B, Ojo O, Adewale O, Olayide I, Emuowhochere R. Biology of glucose metabolization in cancer cells. J Oncol Sci 2017; 3: 45-51.
10) Sun P, Hu J W, Xiong W J, Mi J. miR-186 regulates glycolysis through Glut1 during the formation of cancer-associated fibroblasts. Asian Pac J Cancer Prev 2014; 15: 4245-4250.
11) Gonzalez-Menendez P, Hevia D, Alonso R, Gonzalez-Pola I, Mayo JC, Sainz RM. GLUT1/GLUT4 balance is a marker of androgen-insensitivity in prostate cancer. Eur J Cancer 2016; 61: S41.
12) Fan R, Hou WJ, Zhao YJ, Liu SL, Qiu XS, Wang EH, Wu GP. Overexpression of HPV16 E6/E7 mediated HIF-1α upregulation of GLUT1 expression in lung cancer cells. Tumour Biol 2016; 37: 4655-4663.
13) Kogo R, How C, Chaudary N, Bruce J, Shi W, Hill RP, Zahedi P, Yip KW, Liu FF. The microRNA-218 survivin axis regulates migration, invasion, and lymph node metastasis in cervical cancer. Oncotarget 2015; 6: 1090-1100.
14) Han G, Fan M, Zhang X. microRNA-218 inhibits prostate cancer cell growth and promotes apoptosis by repressing TPD52 expression. Biochem Biophys Res Commun 2015; 456: 804-809.
15) Zhu Z, Xu Y, Du J, Tan J, Jiao H. Expression of microRNA-218 in human pancreatic ductal adenocarcinoma and its correlation with tumor progression and patient survival. J Surg Oncol 2014; 109: 89-94.

16) Cheng Y, Yang X, Deng X, Zhang X, Li P, Tao J, Lu Q. MicroRNA-218 inhibits bladder cancer cell proliferation, migration, and invasion by targeting BMI-1. Tumour Biol 2015; 36: 8015-8023.

17) Liu Z, Xu Y, Long J, Guo K, Ge C, Du R. microRNA-218 suppresses the proliferation, invasion and promotes apoptosis of pancreatic cancer cells by targeting HMGB1. Chin J Cancer Res 2015; 27: 247-257.

18) Dong Y, Zou J, Su S, Huang H, Deng Y, Wang B, Li W. MicroRNA-218 and microRNA-520a inhibit cell proliferation by downregulating E2F2 in hepatocellular carcinoma. Mol Med Rep 2015; 12: 1016-1022.

19) Gao C, Zhang Z, Liu W, Xiao S, Gu W, Lu H. Reduced microRNA-218 expression is associated with high nuclear factor kappa B activation in gastric cancer. Cancer 2010; 116: 41-49.