miRNA-10a promotes cancer cell proliferation in oral squamous cell carcinoma by upregulating GLUT1 and promoting glucose metabolism

YUAN-HUA CHEN1, YU SONG2, YAN-LING YU2, WEI CHENG3 and XIN TONG3

1Department of Prosthodontics, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing, Jiangsu 210008; 2Department of Orthodontics, Qingdao Stomatological Hospital, Qingdao, Shandong 266001; 3Department of Dental Implantation, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing, Jiangsu 210008, P.R. China

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Abstract. MicroRNA-10a (miRNA-10a) promotes lung cancer; however, to the best of our knowledge, its involvement in other cancer types is unknown. The present study aimed to investigate the role of miRNA-10a in oral cancer. Expression levels of miRNA-10a and glucose transporter 1 (GLUT1) in tumor tissues and adjacent healthy tissues obtained from patients with oral squamous cell carcinoma (OSCC) were detected by reverse transcription-quantitative polymerase chain reaction. Correlation analysis between the expression levels of miRNA-10a and GLUT1 was performed using Pearson’s correlation coefficient. It was identified that miRNA-10a and GLUT1 were upregulated in tumor tissues compared with adjacent healthy tissues of patients with OSCC. Expression levels of miRNA-10a and GLUT1 were positively correlated in tumor tissues but not in adjacent healthy tissues. In addition, miRNA-10a overexpression promoted glucose uptake and upregulated GLUT1 in OSCC cells. Furthermore, GLUT1 overexpression promoted glucose uptake; however, no significant increase in the expression level of miRNA-10a in OSCC cells was detected. Overexpression of miRNA-10a and GLUT1 promoted OSCC cell proliferation, while GLUT1-knockdown inhibited OSCC cell proliferation. GLUT1-knockdown also attenuated the enhancing effect of miRNA-10a overexpression on cancer cell proliferation. Therefore, miRNA-10a may promote cancer cell proliferation in OSCC by upregulating GLUT1 and promoting glucose metabolism.

Introduction

As a type of malignancy that develops in the hard palate, lips, the anterior two-thirds of the tongue, the upper and lower alveolar ridges, buccal mucosa, retromolar trigone, sublingual region and floor of the mouth, oral cancer affected >0.0001% of people between 2000 and 2010 in the USA (1,2). The incidence rate of oral cancer has exhibited an increasing trend in recent years (3). The occurrence of oral cancer has been demonstrated to be significantly associated with smoking, alcohol drinking, poor diet, poor oral hygiene and human papilloma virus infection (4). However, to the best of our knowledge, the molecular mechanism of the pathogenesis of oral cancer remains unknown (5), which hinders the development of effective treatment strategies.

Tumor cells are characterized by abnormally accelerated energy metabolism (6). Therefore, inhibition of energy metabolism is considered a promising treatment target for cancer therapy (7). Glucose transporter 1 (GLUT1), also termed facilitated glucose transporter member 1, is a uniporter protein that facilitates the transport of glucose to mammalian cells. A number of studies have reported that GLUT1 is abnormally upregulated in human cancer and promotes cancer development and progression by regulating cancer cell glucose metabolism (8). GLUT1 overexpression promotes glucose uptake and upregulates GLUT1 in OSCC cells. Furthermore, GLUT1 overexpression promoted glucose uptake; however, no significant increase in the expression level of miRNA-10a in OSCC cells was detected. Overexpression of miRNA-10a and GLUT1 promoted OSCC cell proliferation, while GLUT1-knockdown inhibited OSCC cell proliferation. GLUT1-knockdown also attenuated the enhancing effect of miRNA-10a overexpression on cancer cell proliferation. Therefore, miRNA-10a may promote cancer cell proliferation in OSCC by upregulating GLUT1 and promoting glucose metabolism.
Human materials and cell lines. Tumor tissue and adjacent healthy tissue samples were obtained from 52 patients with OSCC who were treated at Nanjing Stomatological Hospital (Nanjing, China) between July 2014 and July 2018. The inclusion criteria were as follows: i) Patients were diagnosed with OSCC by pathological biopsies; ii) patients with complete medical records; iii) patients with no history of another type of malignancy; and iv) patients provided written informed consent. The exclusion criteria were as follows: i) Patients who had been diagnosed with multiple diseases; and ii) patients who had received treatment prior to admission at Nanjing Stomatological Hospital. In total, the present study included 29 males and 23 females, with an age range of 33-65 years and a mean age of 45.3±4.4 years. The current study was approved by the Ethics Committee of Nanjing Stomatological Hospital (Nanjing, China).

The OSCC cell lines SCC090 and SCC25 were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in Eagle's Minimum Essential Medium (ATCC) containing 2 mM L-glutamine (Sangon Biotech Co., Ltd., Shanghai, China) and 10% fetal bovine serum (FBS, Sangon Biotech Co., Ltd.) at 37°C with 5% CO₂.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA Purification kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was used to extract total RNA from tumor tissues, adjacent healthy tissues and in vitro cultured cells. For miRNA extraction, TaqMan miRNA Isolation kit (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used. RevertAid RT Reverse Transcription kit for total RNA reverse transcription (Termo Fisher Scientific, Inc.) and TaqMan microRNA Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for miRNA reverse transcription. qPCR was performed using a Luna® Universal One-Step RT-qPCR kit (catalog no. E3005; New England Biolabs, Inc., Ipswich, MA, USA) or miScript SYBR green AccuPrime™ GO UV/Vis Microplate Spectrophotometer (Thermo Fisher Scientific, Inc.).

Glucose uptake assay. Glucose uptake abilities were measured by a glucose uptake assay 24 h after transfection. A total of 6x10⁵ cells were harvested and washed twice with Krebs-Ringer-HEPES (KRH) buffer (25 mM Hepes, pH 7.4, 120 mM NaCl, 1.2 mM MgSO₄, 5 mM KCl 1.3 mM CaCl₂ and 1.3 mM KH₂PO₄) supplemented with 1 µCi [3H]-2-deoxyglucose (PerkinElmer, Inc., Waltham, MA, USA). Cells were cultured at 37°C in a 5% CO₂ incubator, followed by the addition of 10 µl CCK-8 solution at 24, 48, 72 and 96 h. Subsequently, cells were cultured for a further 6 h and optical density values at 450 nm were measured using a Fisherbrand™ accuSkan™ GO UV/VIS.

Cell proliferation assay. Cell proliferation was evaluated using Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology, Haimen, China) 24 h after transfection. Briefly, cell suspensions with a cell density of 4x10⁴ cells/ml were prepared in Eagle's Minimum Essential Medium containing 2 mM L-glutamine and 10% FBS, and cells were transferred to a 96-well plate with 0.1 ml cell suspension in each well. Cells were cultured at 37°C in a 5% CO₂ incubator, followed by the addition of 10 µl CCK-8 solution at 24, 48, 72 and 96 h. Subsequently, cells were washed with a further 6 h and optical density values at 450 nm were measured using a Fisherbrand™ accuSkane GO UV/Vis.

Total protein extraction and western blot analysis. The effect of miRNA-10a on GLUT1 expression was detected by western blot analysis. A Total Protein Extraction kit (catalog no. NBP2-37853; Novus Biologicals, Ltd., Cambridge, UK) was used to extract total protein from cells, according to the manufacturer's protocol. Electrophoresis was performed to separate denatured proteins using 10% SDS-PAGE gel with 20 µg protein per lane. Following transfer to PVDF membranes, the membranes were blocked with 5% non-fat milk for 2 h at room temperature. Western blot analysis was performed by incubation with rabbit anti-human GLUT1 (1:1,500; catalog no. ab15309; Abcam, Cambridge, UK) and rabbit anti-human GAPDH (1:1,300; catalog no. ab8245; Abcam) at 4°C overnight. The membranes were then incubated with goat anti-rabbit IgG-horseradish peroxidase secondary antibody (1:1,000; catalog no. MBS435036; MyBioSource, San Diego, CA, USA) at room temperature for 2 h. An ECL™ Western Blotting Analysis system (Sigma-Aldrich; Merck KGaA) was used to develop signals. Signals were normalized using Image J v1.46 software (National Institutes of Health, Bethesda, MD, USA).
Statistical analysis. All experiments were performed three times. Data are presented as the mean ± standard deviation and were processed using GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA). Correlation analysis between the expression levels of miRNA-10a and GLUT1 was performed using Pearson's correlation coefficient. Comparisons of the expression levels of miRNA-10a and GLUT1 between tumor tissues and adjacent healthy tissues were performed using a paired Student's t-test. Comparisons among multiple groups were performed by one-way analysis of variance followed by Tukey's test. *p<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of miRNA-10a and GLUT1 were upregulated in tumor tissues compared with adjacent healthy tissues. Expression levels of miRNA-10a and GLUT1 in tumor tissues and adjacent healthy tissues were measured by reverse transcription-quantitative polymerase chain reaction. Data are presented as the mean ± standard deviation. *p<0.05. miRNA-10a, microRNA-10a; GLUT1, glucose transporter 1.

miRNA-10a overexpression upregulates GLUT1 in the OSCC SCC090 and SCC25 cell lines. To further investigate the association between miRNA-10a and GLUT1, miRNA-10a mimic and a GLUT1 expressing vector were transfected into SCC090 and SCC25 OSCC cell lines, followed by the
miRNA-10a overexpression promotes glucose uptake and cell proliferation via GLUT1. The expression levels of GLUT1 in SCC090 and SCC25 cells transfected with GLUT1 siRNA were significantly decreased compared with the control and negative control cells (Fig. 4). Compared with the control and negative control cells, overexpression of miRNA-10a and GLUT1 significantly promoted glucose uptake, while GLUT1-knockdown significantly inhibited glucose uptake (P<0.05; Fig. 5A). In addition, GLUT1-knockdown significantly attenuated the enhancing effects of miRNA-10a overexpression on glucose uptake (P<0.05). Furthermore, GLUT1 overexpression significantly promoted the proliferation of SCC090 and SCC25 cells, while GLUT1-knockdown significantly inhibited the proliferation of SCC090 and SCC25 cells (P<0.05; Fig. 5B). Additionally, GLUT1-knockdown significantly attenuated the enhancing effects of miRNA-10a overexpression on cell proliferation (P<0.05).
A previous study characterized miRNA-10a as an oncogenic miRNA in lung cancer (14); however, to the best of our knowledge, the role of miRNA-10a in other human diseases remains unknown. The present study demonstrated that miRNA-10a is also likely an oncogenic miRNA in OSCC, which is a major type of oral cancer that accounted for <95% of oral cancer cases worldwide in recent years (4,5). The role of miRNA-10a in the proliferation of OSCC cells is likely achieved via an upregulation of GLUT1 and accelerated glucose uptake.

An upregulation of GLUT1 is frequently observed during the development of different types of human cancer, including OSCC (16). Overexpression of GLUT1 promotes glucose metabolism, which provides energy for cancer cell division and proliferation (17). Therefore, inhibition of GLUT1 is considered as a potential therapeutic target for the treatment of different types of cancer (18). Upregulation of GLUT1 also promotes the growth of oral tumors (19). Consistent with previous studies, the present study demonstrated that the expression level of GLUT1 was significantly higher in tumor tissues compared with adjacent healthy tissues of patients with OSCC.

miRNAs serve key roles in the regulation of glucose metabolism in cancer cells (20,21). As an oncogenic miRNA, miRNA-10a is upregulated in lung cancer (14); however, to the best of our knowledge, its expression pattern in OSCC remains unknown. The present study demonstrated that miRNA-10a was upregulated in tumor tissues compared with adjacent healthy tissues of patients with OSCC. In addition, in vitro cell experiments revealed that miRNA-10a promoted cancer cell proliferation and glucose uptake. Therefore, miRNA-10a may serve an oncogenic role in OSCC by upregulating glucose uptake and accelerating cell proliferation.

The expression of GLUT1 is regulated by miRNAs during cancer progression (13). The present study identified a significant positive correlation between the expression levels of miRNA-10a and GLUT1 in tumor tissues. The in vitro experiments revealed that overexpression of miRNA-10a could significantly mediate the upregulation of GLUT1, while overexpression of GLUT1 did not significantly alter the expression of miRNA-10a. Furthermore, silencing of GLUT1 with siRNA significantly attenuated the enhancing effects of miRNA-10a overexpression on cancer cell proliferation and glucose uptake. Therefore, miRNA-10a may promote cancer cell proliferation and glucose uptake in OSCC by serving as an upstream activator of GLUT1. Previous studies have also reported that miRNAs participate in the growth, development and progression of cancer by regulating the expression of GLUT1 (13,22). In summary, the current study identified a novel miRNA regulator of GLUT1 in cancer biology.
The present study is limited by the small sample size. Therefore, studies with a larger sample size and further correlation analyses are required. Notably, there is no promising binding site of miRNA-10a in GLUT1 based on a local blast analysis. In addition, the correlation between miRNA-10a and GLUT1 expression levels was not significant in adjacent normal tissues. Therefore, miRNA-10a may be not able to directly regulate GLUT1 and the interaction between miRNA-10a and GLUT1 may be mediated by certain pathological factors, such as tumor suppressive or oncogenic pathways. The present study failed to perform miR-10a silencing experiments due to a low knockdown efficiency; therefore, this should be improved in future studies.

In conclusion, miRNA-10a was identified to be upregulated in OSCC. miRNA-10a may promote cancer cell proliferation and glucose uptake in OSCC by acting as an upstream activator of GLUT1.

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Availability of data and materials
The analyzed datasets generated during the present study are available from the corresponding author on reasonable request.

Authors’ contributions
YC and WC designed experiments. YC and YS performed experiments. YY and XT analyzed data. WC drafted the manuscript and all authors approved this manuscript.

Ethics approval and consent to participate
The present study was approved by the Ethics Committee of Nanjing Stomatological Hospital (Nanjing, China). All patients provided written informed consent prior to their inclusion in the study.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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