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Silencing PFKP inhibits starvation-induced autophagy, glycolysis, and epithelial mesenchymal transition in oral squamous cell carcinoma

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ABSTRACT

The tumor starvation microenvironment plays a pivotal role in the malignant progression of cancer, which is closely related to autophagy, glycolysis, and epithelial mesenchymal transition (EMT). Nevertheless, the underlying mechanisms of the starvation-mediated malignant phenotype are still not well documented. In this study, we aimed to investigate the effect of starvation on glycolysis, autophagy, and EMT in OSCC and to further elucidate the key metabolic modulator. The results showed that starvation can induce autophagy, EMT, and enhanced glycolysis in OSCC cells. We determined that the expression of the key glycolytic enzyme phosphofructokinase-platelet (PFKP) obviously increased under starvation conditions and that PFKP knockdown inhibited starvation-mediated glycolysis, autophagy and EMT in OSCC cells. Moreover, we confirmed that PFKP knockdown inhibited OSCC xenograft growth in vivo. In addition, PFKP expression was significantly increased in OSCC patients and its upregulation was associated with the presence of tumor pathological differentiation and lymph node metastasis. Taken together, our findings demonstrate that PFKP is necessary for starvation-mediated autophagy, glycolysis, and EMT, thereby promoting the malignant progression of OSCC.

1. Introduction

Oral squamous cell carcinoma (OSCC) is life-threatening and is the most common type of cancer in the head and neck region [1]. Over the past 30 years, despite the improvement of comprehensive therapeutic strategies, the current understanding of the prognostic factors for oral cancer is still insufficient [2,3]. Recently, many studies have indicated that the malignant progress of cancer is closely associated with the tumor microenvironment, including nutrient deprivation due to poor vascularization [4,5]. Nutrient deprivation may induce autophagy, EMT, and enhanced glycolysis, which in turn promotes the malignant progression of cancer cells.

Autophagy, an evolutionarily conserved, catabolic process, can serve to degrade damaged organelles and misfolded proteins, which in turn helps regulate aerobic glycolysis and provides an alternative energy source to maintain cell homeostasis [6,7]. Several studies have confirmed that autophagy can be activated for adaptation to starvation [8,9]. The epithelial-mesenchymal transition (EMT) is a biological process by which epithelial cells lose cell-to cell junctions and polarity, leading to a change from the epithelial to the mesenchymal phenotype [10]. Increasing evidence demonstrates that EMT is a crucial mechanism contributing to tumor migration and invasion [11,12]. Aerobic glycolysis substitutes for the preferential use of glycolysis to provide energy, despite the presence of oxygen, and has become a hallmark of the cancer phenotype [13]. The platelet isoform of phosphofructokinase (PFKP) is the second main rate limiting enzyme mediating glycolytic rate and aiding tumor cells to adapt to the unfavorable tumor microenvironment [14]. Most cancers express a high level of PFKP, which is associated with prognosis, and the autophagy is involved in the regulation of aerobic glycolysis [15,16]. However, the relationship between PFKP expression and OSCC progression, as well as the effect of nutrient deprivation on the biological behavior of cancer cells and the underlying mechanisms are not well documented for OSCC.

In this study, we aimed to explore the effect of starvation on autophagy, EMT, and glycolysis in OSCC cancer cells, and to further investigate the potential mechanism of the key metabolic molecule PFKP

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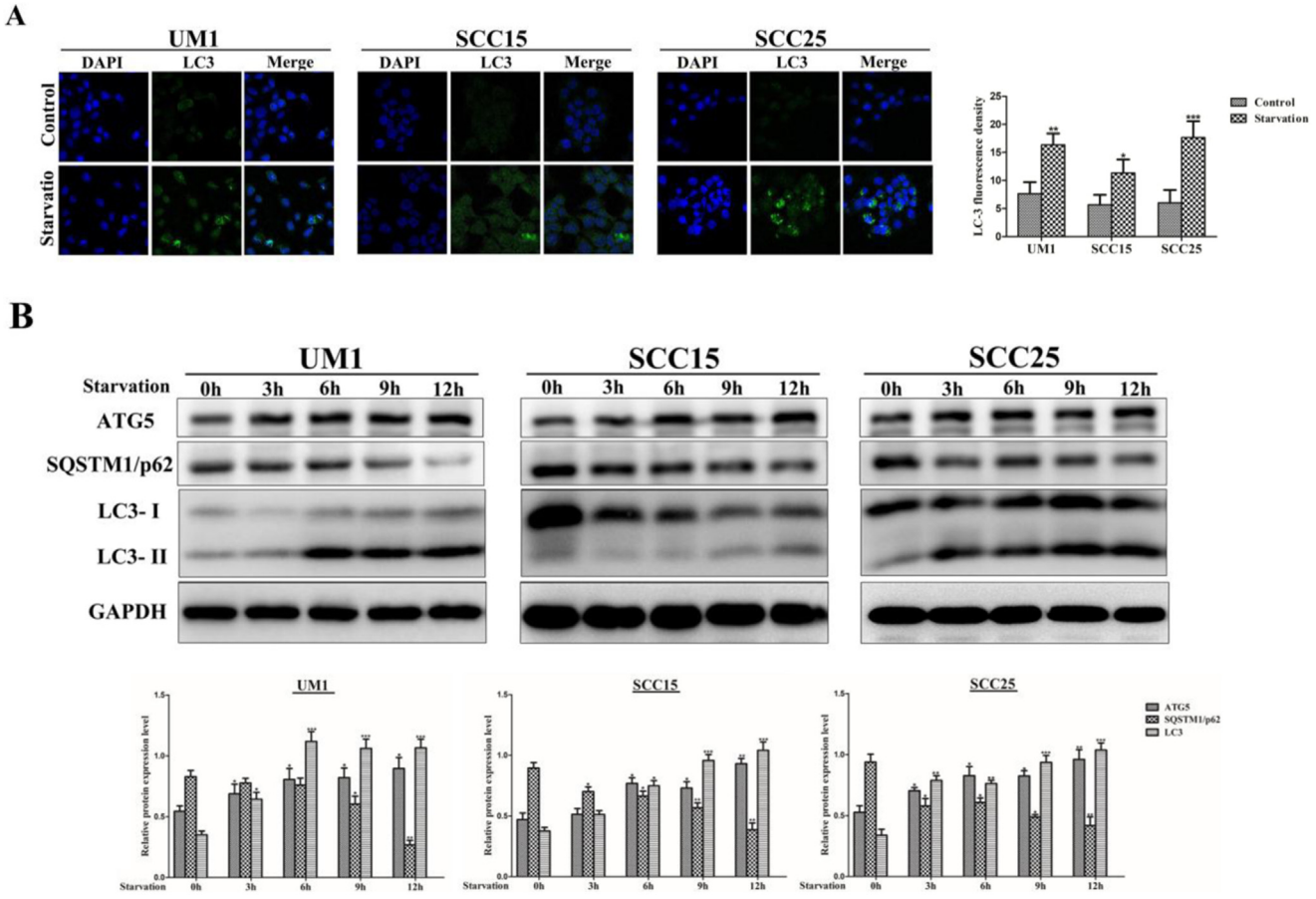

Fig. 1. Effects of starvation on OSCC cell autophagy, glycolysis, proliferation, migration, and invasion. (A) Immunofluorescence analysis was used to confirm the expression level of LC3 in UM1, SCC15, and SCC25 cells with or without starvation. (B) The expression of autophagy related protein ATG5, SQSTM1/p62 and LC3 in OSCC cells were evaluated by western blot under starvation for different periods of time and the quantitative analysis of ATG5, SQSTM1/p62 and LC3 are presented correspondingly. (C-D) Relative change in glucose consumption (C) and lactate production levels (D) were shown in OSCC cells under starvation at different periods of time. (E) Starvation increased the expression of Glut-1 and PFKP in OSCC cells while HK-II and PKM2 levels did not alter, as measured by western blot. The Glut-1 and PFKP levels were estimated as a ratio to the levels of GAPDH for all three cell lines. (F) OSCC growth curves were determined using a CCK-8 assay. (G-H) Representative results of migratory (G) and invasive (H) ability and corresponding quantitative analysis are shown for all three cells. Images are presented at 100 × magnification. (I) E-cadherin, vimentin, Snail, and Slug expressions in OSCC cells were assessed by western blot and the corresponding quantitative data is presented as a ratio to the levels of GAPDH in OSCC cells. Values are the mean ± SD from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

that regulates glycolysis, autophagy, and EMT in OSCC under starvation.

2. Material and methods

2.1. Patients and specimens

After obtaining informed consent from all patients, 90 OSCC patients were enrolled and 20 matching adjacent noncancerous tissues (ANCT) were collected from the Hospital of Stomatology at the Guanghua School of Stomatology of Sun Yat-sen University, between October 2009 and October 2015. None of the enrolled patients received chemotherapy or radiotherapy prior to surgery. Clinicopathological staging of the tumor was determined according to the American Joint Committee on Cancer Classification Criteria. This study was approved by the ethics committee of the Hospital of Stomatology, Sun Yat-Sen University.

2.2. Cell lines and culture

The human oral squamous cell carcinoma cell lines UM1, SCC15, and SCC25 were grown in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12, Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA) and supplemented with 400 ng/mL Glucocorticoid (Sigma-Aldrich) under humidified conditions with 5% CO_2 at 37 ℃. For starvation treatment, cells were incubated in glutamine-free DMEM without FBS (Gibco, USA).

2.3. Western blot analysis

Cells were harvested and lysed with RIPA buffer and 0.1% PMSF (Cwbio, China). The protein concentration was measured with a BCA protein assay kit (Cwbio, China). Equal amounts of samples were then separated by SDS-PAGE and transferred to PVDF membranes (Millipore, USA). After being blocked with 5% bovine serum albumin (Cwbio, China), incubated sequentially with specific primary antibodies overnight at 4 ℃, and being probed with secondary antibodies, the bands were visualized by enhanced chemiluminescence (Millipore, USA). The primary antibodies used are as follows: microtubule-associated protein 1 light chain 3 (LC3), sequestosome 1 (SQSTM1/p62), autophagy-related gene 5 (ATG5), Hexokinase II (HKII), PFKP, pyruvate kinase M2 (PKM2), Glucose transporter-1 (Glut-1), E-cadherin, vimentin, Snail, Slug, and GAPDH (Cell signaling, USA).