Original Article

SDHB reduction promotes oral lichen planus by impairing mitochondrial respiratory function

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Background: Oral lichen planus (OLP) is a type of chronic inflammatory disorder, which represents a potential risk of malignant transformation. Understanding the mechanism of OLP-related malignant transformation could reduce the risk of cancer. Accumulating evidence indicates that the expression of succinate dehydrogenase enzyme B (SDHB) is associated with the carcinogenesis of oral squamous cell carcinoma (OSCC). However, the function and underlying mechanism of SDHB in OLP remains unknown.

Methods: In this study, we examined the expression of SDHB in tissues from OLP patients and normal oral mucosa (NOM) through immunohistochemical (IHC) staining, quantitative reverse transcription polymerase chain reaction (qRT-PCR), and western blot (WB). Adenosine triphosphate (ATP) assay, reactive oxygen species (ROS) assay, mitochondrial membrane potential (MMP) assay, and glucose uptake assay were used to explore the function of SDHB in mitochondrial injury and bioenergetic changes in OLP cell model and SDHB-overexpressing cells.

Results: In current study, we found that the messenger RNA (mRNA) and protein expression of SDHB was significantly decreased in OLP patients, accompanied by the accumulation of succinate. In the lipopolysaccharide (LPS) or $CoCl_2$-stimulated OLP cell model, the expression of SDHB was decreased along with treatment time and concentration. Mechanistically, decreased SDHB enhanced hypoxia-inducible factor (HIF)-1α activity, induced mitochondrial injury, bioenergetic changes, and cytokine release. Overexpression of SDHB could reverse the above biological process and switch bioenergetic metabolism during OLP process.

Conclusions: Our study suggests that SDHB reduction promotes OLP by impairing mitochondrial respiratory function.

Keywords: Succinate dehydrogenase enzyme B (SDHB); oral lichen planus (OLP); mitochondrial respiratory function; hypoxia-inducible factor-1α (HIF-1α); glycolytic metabolism

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Introduction

Oral lichen planus (OLP) is a chronic inflammatory disorder that affects oral mucosa, with various clinical manifestations including papular, reticular, plaque-like, ulcerative, and atrophic lesions (1,2). Despite the lack of reliable epidemiological data, OLP is thought to be common, affecting about 1.5% of all people (3). Nowadays, the pathogenesis of OLP remains unclear, and microbial dysbiosis, innate and adaptive immunity and inflammation has been proposed to play crucial roles (4-6). The lesions of OLP lesions are chronic, rarely spontaneously resolve (7), and they always cause severe pain, morbidity, and undergo

malignant transformation (8,9). OLP has been suggested as a potential a precancerous event for oral squamous cell carcinoma (OSCC), the common oral cancer subtype that comprises over 90% of cases (10). OLP has diverse molecular pathogenic mechanisms according to different etiologic factors and genotoxic stimuli, such as exposure to toxicity, viral infections, and metabolic factors (10,11). Therefore, there is an urgent need to understand the mechanisms underlying the OLP-related malignant transformation and provide targets for OLP progression.

Succinate dehydrogenase enzyme (SDH), the heterotetrameric protein with high conservation level, comprises 4 subunits: SDHA/B/C/D, which is responsible for catalyzing succinate oxidation to fumarate during tricarboxylic acid (TCA) cycle (12,13). Of them, SDHA and SDHB have catalytic effects, whereas the others assist in anchoring the former 2 to the mitochondrial membrane (14-16). Compared to the other 3 subunits, *SDHB* mutations are more aggressive and have a higher metastasis rate (17,18). The damage of SDHB function induces mitochondrial dysfunction, rewired metabolism, and pseudohypoxic signaling, all of which play important roles in promoting tumor progression. Deactivation of SDHB is extensively suggested to be associated with cancer malignancy (19,20), whereas its somatic mutations are frequently related to pheochromocytoma, paraganglioma, renal cell carcinoma, and gastrointestinal tumors (21,22). Apart from mutation inactivation, decreased expression of SDH subunits can be detected within multiple cancers including that of the brain and breast (23,24). Further studies show that intratumoral hypoxia, which is in favor of cancer cell proliferation, invasion and metastasis, inhibits the expression of SDHB, and the decreased expression of SDHB also promotes the hypoxia-induced injury (25,26). Meanwhile, as the mediation between oxidative phosphorylation and electron transport, SDHB regulates mitochondrial ROS production and drives macrophage inflammatory, including interleukin (IL)-1β, IL-18 release (27). However, the molecular mechanisms leading to SDH impairment in precancerous lesions remain largely unknown. Besides, impairment of SDHB activity leads to accumulation of the succinate, which could drive aberrant activation of transcription factors and global epigenetic reprogramming (28,29). Recently, metabolic alterations have been suggested to be related to OLP progression (30,31). Therefore, therapeutic strategies targeting mitochondrial metabolism might have important implications for the treatment of OLP progression.

In current study, we found that altered SDHB expression was closely associated with OLP progression. Mechanistically, decreased SDHB led to accumulation of succinate, which then promoted hypoxia-inducible factor (HIF)-1α activation and impaired mitochondrial respiration. Our study suggests that SDHB overexpression is the possible treatment for OLP cases showing decreased SDHB expression. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5999/rc).

Highlight box

Key findings

- SDHB reduction promotes OLP by impairing mitochondrial respiratory function.

What is known and what is new?

- SDHB expression is associated with the malignant transformation from OLP to OSCC.
- SDHB expression was indeed significantly reduced with OLP occurrence, accompanied by the change of cell biogenetics to glycolytic metabolism from aerobic respiration, providing the plausible explanation for possible cellular deterioration and transformation to cancer.

What is the implication, and what should change now?

- SDHB is an effective indicator of OLP-induced carcinogenesis and may be a potential therapeutic target.

Methods

Patients and tissue specimens

This work involved a total of 23 OLP samples obtained from Huashan Hospital, Fudan University during 2018 to 2020. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Research Ethics Committee of Huashan Hospital, Fudan University (No. 2019-589) and informed consent was taken from all the patients. The information of study participants was shown in Table S1.

Cells and culture

Under sterile conditions, samples of reticular lesion tissue measuring 0.6 cm × 1.0 cm were collected from OLP cases. Later, sterile phosphate-buffered saline (PBS) containing 1% penicillin-streptomycin (PS; Gibco, Waltham, MA, USA) was added to immerse the samples for removing

Table 1 The sequences of qRT-PCR primers used in this study

Gene	Forward primer	Reverse primer
SDHB	5'-CATCAATGGAGGCAACACTCT-3'	5'-CAAATCGGGAACAAGATCCTT-3'
GLUT1	5'-CACCACCTCACTCCTGTTACT-3'	5'-CATCCAAACCTCCTACCCT-3'
PKM2	5'-CCATTGGTCATCAGGTTTCT-3'	5'-TTCGGTGGGACTAAATTCTG-3'
MMP9	5'-GCTGGGCTTAGATCATTCCTC-3'	5'-ATTCACGTCGTCCTTATGCAA-3'
NLRP3	5'-TGGACTATTTCCCCAAGATTG-3'	5'-CCCGATGACAGTTCTCAATG-3'
ASC	5'-CCACCAACCCAAGCAAGATG-3'	5'-CTCCAGGTCCTCCACCAGGTAG-3'

qRT-PCR, quantitative reverse transcription polymerase chain reaction; *SDHB*, succinate dehydrogenase enzyme B; *GLUT1*, glucose transporter type 1; *PKM2*, pyruvate kinase M2; *MMP9*, matrix metallopeptidase 9; *NLRP3*, NOD-like receptor family pyrin domain containing 3; *ASC*, apoptosis-associated speck-like protein containing a CARD.

blood. Thereafter, keratinocyte serum-free medium (KSFM) was added to culture OLP type-I keratinocytes *in vitro*. Cells of 3–4 passages were harvested for further analysis. Meanwhile, HaCAT cells were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China) and cultivated with Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA) under 5% concentration of CO_2 and 37 ℃ conditions. To mimic the hypoxic and inflammatory processes in epidermal injury, we stimulated HaCAT cells with 5 μg/mL LPS.

Western blot (WB) assay

The cell lysis buffer that contained protease/phosphorylase inhibitors was added to extract total cellular protein. Thereafter, 5% defatted milk supplemented within tris-buffered saline-Tween 20 (TBST) was supplemented to block membranes, followed by primary antibody incubation, including SDHB (ab178423; Abcam, Cambridge, MA, USA), hexokinase (HK)1 [Cell Signaling Technology (CST), 2024; CST, Danvers, MA, USA], HK2 (CST, 2867; CST), HIF-1α (ab51608; Abcam), matrix metallopeptidase 9 (MMP9) (ab194316; Abcam), pyruvate kinase M2 (PKM2) (ab150377; Abcam), glucose transporter type 1 (GLUT1) (ab115730; Abcam), apoptosis-associated speck-like protein containing a CARD (ASC) (AG-25B-0006PF-C100; Adipogen, San Diego, CA, USA), NOD-like receptor family pyrin domain containing 3 (NLRP3) (AG-20B-0014-C100; Adipogen), goat anti-mouse horseradish peroxidase (HRP) (ab97023; Abcam) and goat anti-rabbit HRP (ab6721; Abcam). Membranes were rinsed with TBST prior to further secondary antibody incubation, with β-actin as the endogenous reference.

RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

This work adopted Trizol reagent (Invitrogen, USA) for extracting total RNAs in line with specific instructions. *Table 1* displays the sequences of all primers. Total RNA (1 mg) was weighed to synthesize complementary DNA (cDNA) with the cDNA synthesis kit (Takara, Madison, WI, USA). Finally, SYBR Supermix (Takara) was employed for qRT-PCR assay.

Immunohistochemical (IHC) assay

Tissues were rinsed with PBS thrice (5 min each) and then immersed for 15 minutes in pre-chilled alcohol prior to blocking by PBS that included 0.1% Triton X-100 and 1% bovine serum albumin (BSA) under ambient temperature for 1 hour. Thereafter, the primary antibody was added to incubate tissues at 4 ℃ overnight. Tissues were then rinsed with PBS thrice (10 min each), followed by 1 hour of incubation using SDHB (ab178423), anti-rabbit immunoglobulin G (IgG) under ambient temperature. Then, tissues were rinsed in PBS thrice (10 min each), followed by 4 minutes 100 μl DAB substrate solution incubation (Ventana/Roche; Tucson, AZ, USA). Last, tissues were stained by hematoxylin and dehydrated with increasing ethanol concentrations. Afterwards, the Fluoromount Aqueous Mounting Medium (F4680; Sigma, St. Louis, MO, USA) was added to seal the coverslip. The laser scanning confocal unit (Zeiss LSM 710; Carl Zeiss, Jena, Germany)

was utilized to analyze each slice.

Firefly luciferase adenosine triphosphate (ATP) assay

An ATP Bioluminescence Assay Kit CLSII (Roche Applied Science, Mannheim, Germany) was employed for measuring total ATP in cells in line with specific protocols.

Mitochondrial membrane potential (MMP) assay

A JC-10 assay kit (40752ES60; Yeasen Biotechnology, Shanghai, China) was utilized to monitor MMP. Briefly, JC-10 was added to incubate cells for a 30-min period, followed by rinsing with a buffer twice. The fluorescence was detected under flow cytometry (FCM) using a CytoFLEX (Beckman Coulter, Brea, CA, USA).

Reactive oxygen species (ROS) measurement

After 48 hours, dichloro-dihydro-fluorescein diacetate (DCFH-DA; 10 μM) was added into cells within the phenol red-free culture medium (50101ES01; Yeasen Biotechnology) for a 30-min period. After staining, FCM was conducted to analyze cells with the CytoFLEX (Beckman Coulter).

Glucose uptake assay

The glucose uptake was detected by Cell Meter 2-NBDG kit (AAT BioQuest, Sunnvale, CA, USA). After seeding, the cells were treated with glucose-free Krebs-Ringer bicarbonate (KRB) buffer containing 129 mM NaCl, 10 mM HEPES, 5 mM $NaHCO_3$, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, and 1.0 mM $CaCl_2$, together with 0.1% BSA (pH 7.4) for 15 minutes, followed by incubation with the freshly prepared KRB buffer that contained a D-glucose fluorescent analogue 2-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino-2-deoxy-D-glucose (600 mM, 2-NBDG; Molecular Probes Inc., Eugene, OR, USA) and 3.3 Mm glucose for 10–30 min. Finally, the plate reader (Tecan, Männedorf, Switzerland) was utilized to detect fluorescence intensities at the excitation and emission wavelengths of 485 and 530 nm, separately.

Statistical analysis

Data are representative of at least three independent experiments. Results were represented to be means ± standard error of the mean (SEM) and analyzed by one-way analysis of variance (ANOVA) using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Two groups were compared with independent group *t*-tests. *P<0.05, **P<0.01, and ***P<0.001 indicated statistical significance.

Results

SDHB expression decreased and succinate accumulated in OLP patients

In our previous study, we found that TCA cycle was suppressed during the development of OLP (30). SDHB, an important enzymatic constituent in the mitochondrial enzyme SDH (18), is constructively distributed among different species, and we first examined SDHB expression in tissues from OLP patients. By IHC, we found that OLP tissues exhibited significantly reduced SDHB expression compared with normal tissues (*Figure 1A*). SDHB messenger RNA (mRNA) and protein levels were measured among 23 OLP patients by qRT-PCR and WB. Normal oral mucosa (NOM) served as a control. According to *Figure 1B,1C*, OLP samples showed significantly decreased *SDHB* mRNA levels, and WB showed similar results. Expectedly, decreased SDHB levels within OLP samples resulted in damaged succinate activity as well as its later accumulation (*Figure 1D*). Taken together, SDHB showed down-regulation among OLP cases, and it was indicative of certain biological activity in pathology.

SDHB is crucially involved in the prognosis of lipopolysaccharide- (LPS-) and $CoCl_2$-stimulated OLP cell model

OLP is a process in which tissue hypoxia and chronic inflammatory infiltration lead to the deterioration of OLP keratinocytes (32,33). We first stimulated HaCAT cells with LPS and $CoCl_2$ to mimic the hypoxic and inflammatory processes in epidermal injury. Under the stimulation of 5 μg/mL LPS, the expression of SDHB decreased within the prolongation of inflammation time. Under the stimulation of $CoCl_2$ at different concentrations, the expression of SDHB was also reduced within the increase of the degree of hypoxia (*Figure 2A,2B*). To further gain a real picture of OLP development, we isolated OLP keratinocytes. Likewise, after stimulation with LPS and $CoCl_2$ in OLP keratinocytes, we monitored the expression level of SDHB

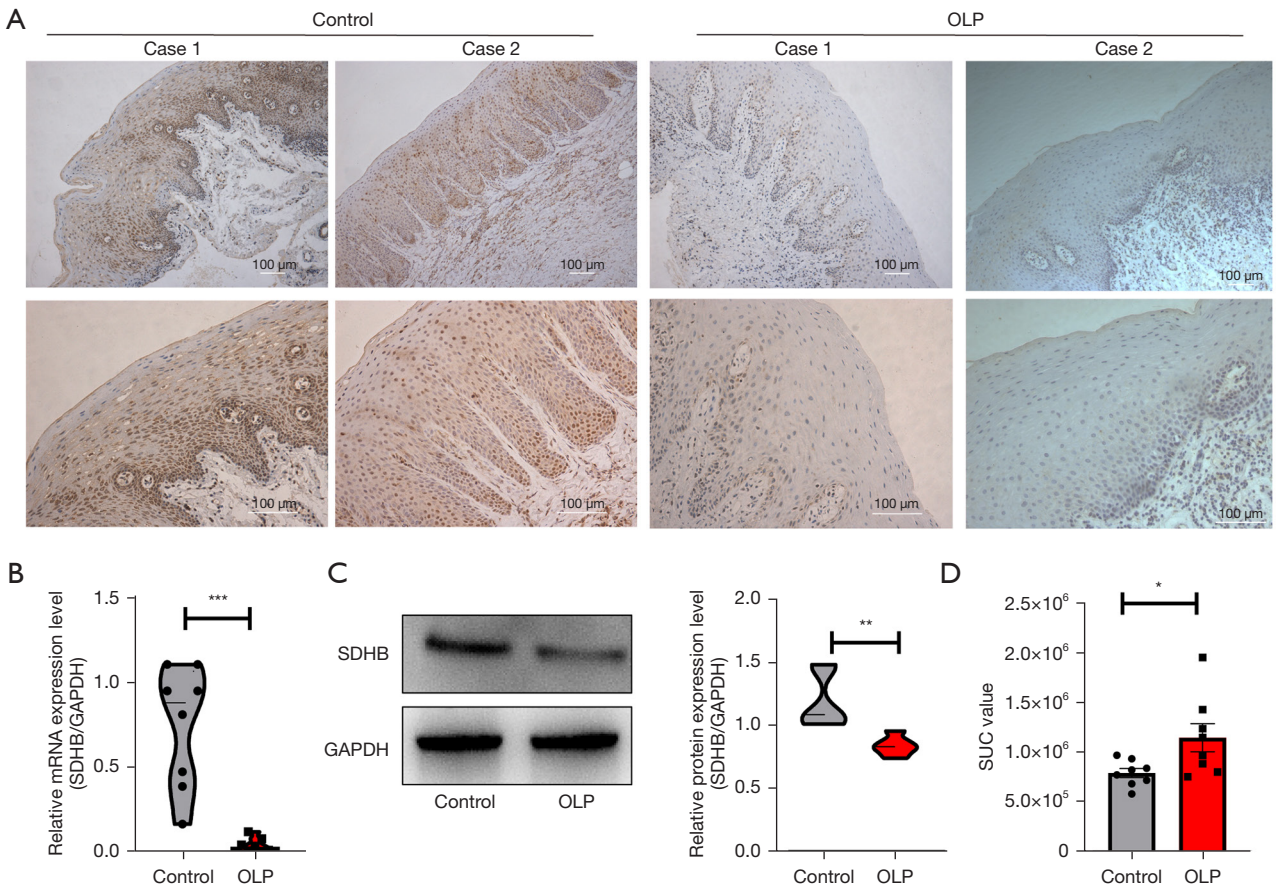

Figure 1 SDHB expression decreased and succinate accumulated in OLP patients. (A) IHC staining of SDHB in OLP patients. Control and OLP indicate the NOM and OLP patient tissues, respectively. (B) Comparison of *SDHB* mRNA levels between NOM and OLP tissues. (C) WB analysis of SDHB expression in NOM and OLP tissues. (D) The expression level of SUC in OLP compared with NOM tissue. Each dot represents a sample. P value was derived from independent sample two tailed *t*-test. The data were presented as mean ± SEM. *P<0.05; **P<0.01; ***P<0.001. OLP, oral lichen planus; mRNA, messenger RNA; SDHB, succinate dehydrogenase enzyme B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IHC, immunohistochemical; NOM, normal oral mucosa; WB, western blot; SUC, succinate; SEM, standard error of the mean.

at different time points or different concentrations. As shown in *Figure 2C,2D*, in the LPS model, 5 μg/mL LPS caused a significant down-regulation of SDHB, reaching the highest inhibitory effect at 24 hours. The same results were observed in the hypoxia model (*Figure 2C,2D*).

SDHB induced mitochondrial injury and bioenergetic changes

SDHB has been identified as an important enzymatic constituent in SDH, which shows extensive expression among different species and is responsible for succinate oxidization into fumarate during TCA cycle together with electron feeding in the mitochondrial respiratory chain to produce ATP (34,35). To evaluate the changes in bioenergetic metabolism caused by reduced SDHB expression during OLP, we assessed the cellular ATP level and ROS level, respectively. The ATP level was detected with the firefly luciferase ATP assay which revealed mild elevation during inflammation and hypoxia compared with the normal group (*Figure 3A*). Since ROS are generated during aerobic respiration, the reduced respiratory activity may thereby decrease ROS generation. To measure whether SDHB decreased mitochondrial respiration, this work

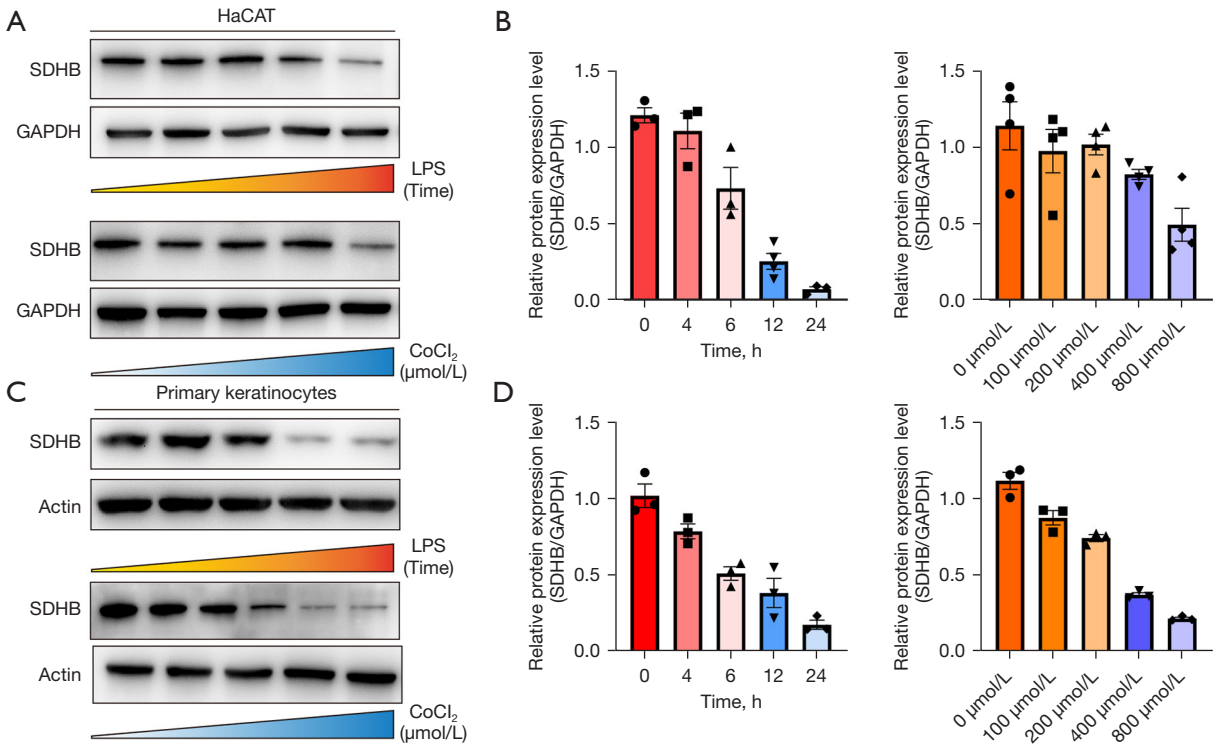

Figure 2 SDHB is crucially involved in the fate decision of LPS and $CoCl_2$-stimulated OLP cell model. (A,B) SDHB expression level detection in HaCAT cells via WB. HaCAT cells were treated with LPS and collected at indicated times (up). HaCAT cells were treated with $CoCl_2$ on different concentrations (down). (C,D) SDHB expression level detection in isolated primary keratinocytes cells via WB. Each dot represents a sample. HaCAT cells were treated with LPS and collected at indicated times (up). HaCAT cells were treated with $CoCl_2$ on different concentrations (down). SDHB, succinate dehydrogenase enzyme B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPS, lipopolysaccharide; OLP, oral lichen planus; WB, western blot.

utilized DCFH-DA, the fluorescent probes, to measure ROS contents. As expected, low staining intensity with DCFH-DA were detected in the inflammatory and hypoxia processes (*Figure 3B,3C*). Based on the above findings, we deduced that SDHB deletion impairs mitochondrial activity while reducing respiratory activity, which was evaluated by calculating MMP (Δψm). It was found that the membrane potential decreased with decreasing SDHB expression, suggesting that SDHB inhibition impairs the progression of the TCA cycle and reduces mitochondrial respiration (*Figure 3D,3E*). The above results imply that changes in SDHB level impair mitochondrial respiration, thus activating and up-regulating cytoplasmic glycolysis to generate ATP in cells. We further examined glucose uptake by adding the fluorescent glucose analog 2-NBDG to the medium to test our hypothesis. The results showed increased glucose uptake as SDHB expression decreased in comparison with normal controls (*Figure 3F*). Collectively, SDHB expression alteration impairs mitochondrial function and induces a switch in bioenergetics to glycolytic metabolism from aerobic respiration.

SDHB enhances HIF-1α and glycolytic metabolism

To further explore our hypothesis, we analyzed the extent of metabolic and inflammatory changes during OLP progression. The expression of genes involved in bioenergy metabolism as well as inflammation, including glycolysis, and inflammatory pathways, was examined (*Figure 4A*). In both models, we first found that the expression of HIF-1α was up-regulated, indicating that reduction of SDHB induces SDH deficiency in cells and changes in oxygen content in the cellular environment, resulting in the stabilization of HIF-1α. At the same time, we found that the expression of HK2 was decreased whereas that of HK1 was greatly elevated (*Figure 4A*). By contrast, inflammasome

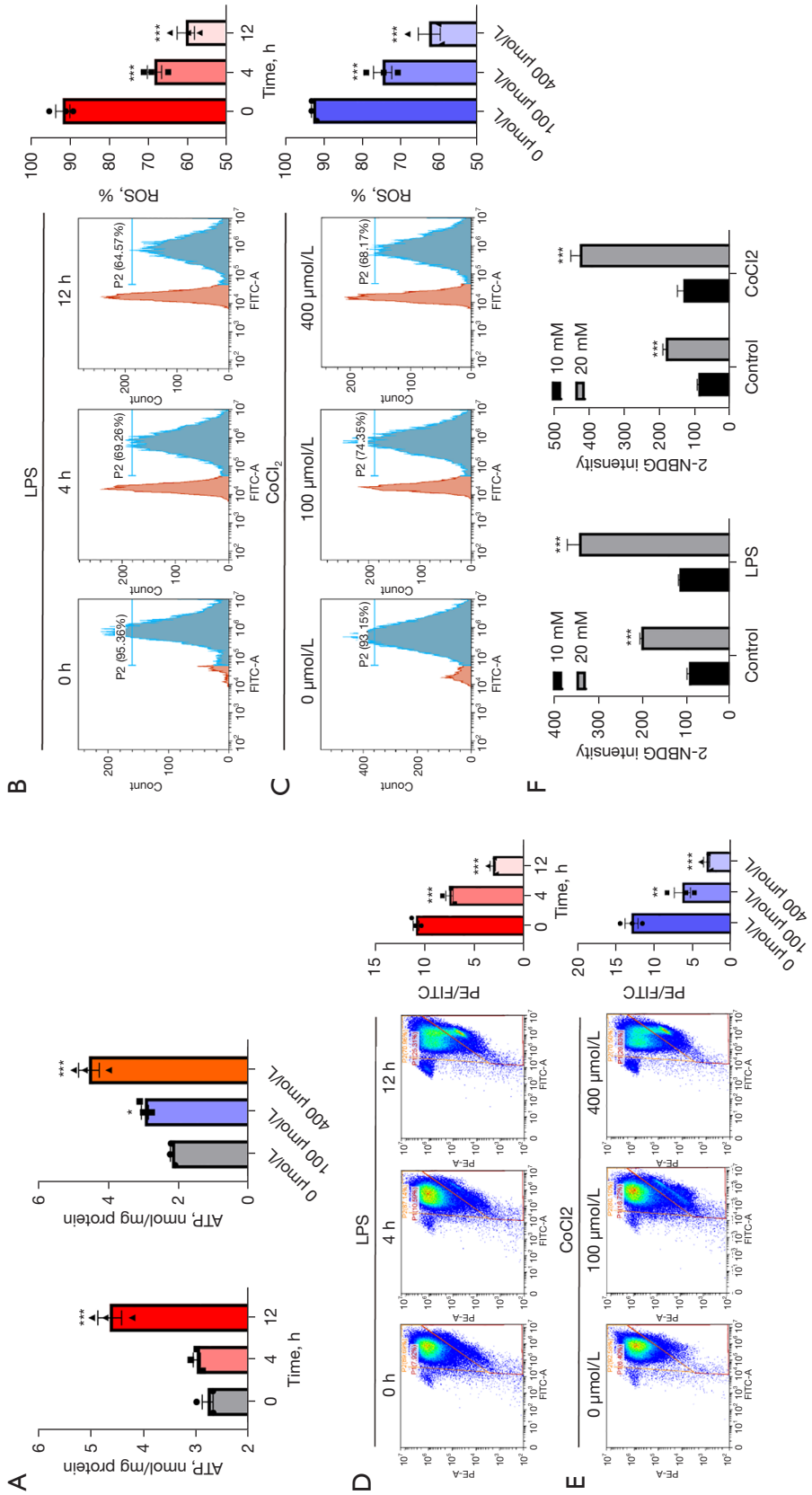

Figure 3 SDHB induces mitochondrial injury and bioenergetic changes. (A) ATP assay detection in OLP cell models. Total extracts prepared from cells as indicated were subjected to an ATP assay using the ATP Bioluminescence Assay Kit CLSII. (B,C) ROS assay detection in OLP cell models. Cells were treated with DCFH-DA probe and analyzed using a FCM. (D,E) MMP assay detection in OLP cell models. Cells were stained with JC-10 and then analyzed using a FCM. (F) Glucose uptake assay of detection in OLP cell models. Cells as indicated were loaded with 2-NBDG and then analyzed using a FCM. Each dot represents a sample. All data shown as mean ± SEM. *P<0.05; **P<0.01; ***P<0.001. ATP, adenosine triphosphate; LPS, lipopolysaccharide; ROS, reactive oxygen species; PE, P-phycoerythrin; FITC, fluorescein isothiocyanate; SDHB, succinate dehydrogenase enzyme B; OLP, oral lichen planus; DCFH-DA, dichloro-dihydro-fluorescein diacetate; FCM, flow cytometer; MMP, mitochondrial membrane potential; SEM, standard error of the mean.

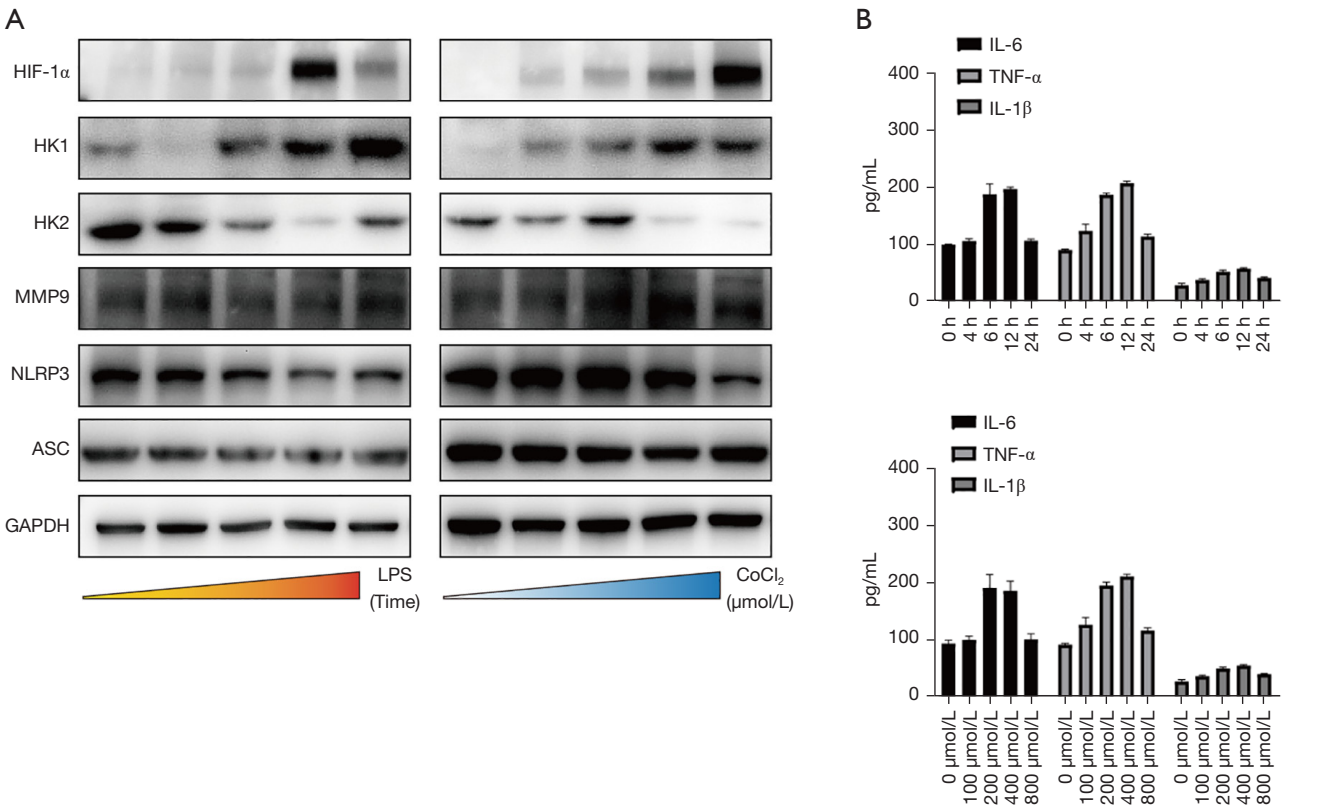

Figure 4 SDHB enhances HIF-1α and promotes glycolytic metabolism. (A) WB analysis of proteins involved in glycolysis and inflammatory signals in OLP cell models. Total proteins isolated from cells were hybridized with antibodies for glycolytic and aerobic respiration enzymes. (B) The cytokine levels of IL-6, TNF-α, and IL-1β were determined by ELISA. HIF, hypoxia-inducible factor; HK, hexokinase; MMP9, matrix metallopeptidase 9; NLRP3, NOD-like receptor family pyrin domain containing 3; ASC, apoptosis-associated speck-like protein containing a CARD; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; SDHB, succinate dehydrogenase enzyme B; WB, western blot; OLP, oral lichen planus; ELISA, enzyme-linked immunosorbent assay.

expression slightly decreased correspondingly, the level of inflammation in this process was only slightly increased, and the inflammatory syndrome gradually subsided over time (*Figure 4B*). These results support that SDHB mediates glycolysis by promoting the stabilization of HIF-1α.

SDHB overexpression switches bioenergetic metabolism during OLP process

To further investigate whether SDHB protein was related to OLP development, we examined how SDHB up-regulation affected OLP cells by adopting overexpression plasmids (*Figure 5A*). To assess how SDHB up-regulation affected bioenergetic metabolism, firefly luciferase assay was conducted to determine ATP levels within cells with SDHB over-expression. Cells with SDHB over-expression showed significantly decreased ATP contents relative to those transfected with vector (*Figure 5B*). To analyze how SDHB up-regulation affected aerobic respiration, the fluorescent probe DCFH-DA was used to measure ROS formation. In contrast, higher staining intensity of DCFH-DA was measured within cells with SDHB over-expression relative to those transfected with vector (*Figure 5C*). The glucose absorption capacity of cells with SDHB over-expression was examined with the fluorescent glucose analog 2-NBDG loading assay. Glucose uptake was slightly reduced in SDHB-overexpressing cells relative to cells transfected with vector and mock cells (*Figure 5D*). Consequently, SDHB overexpression in OLP cells disrupted TCA cycle progression.

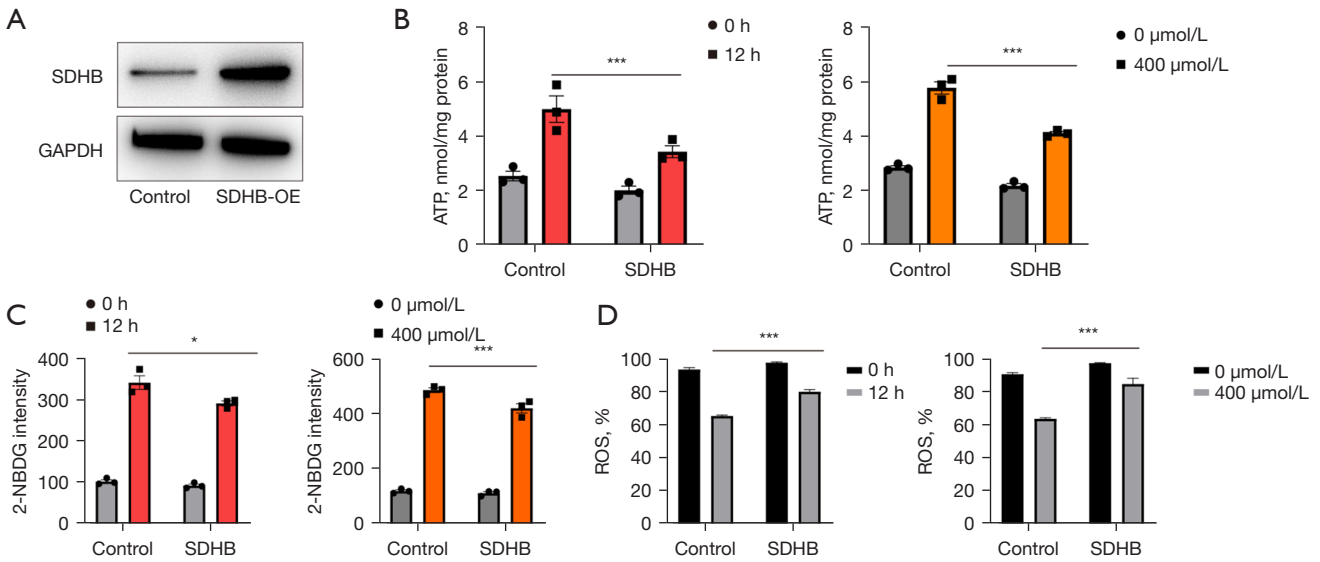

Figure 5 SDHB overexpression switch bioenergetic metabolism during OLP process. (A) WB analysis of SDHB protein in SDHB-overexpression cells. (B) ATP assay of SDHB-overexpressing cells. Total extracts were prepared from cells as indicated, and an ATP assay was performed using the ATP Bioluminescence Assay Kit CLSII. (C) Glucose uptake assay of SDHB-overexpressing cells. Cells as indicated were loaded with 2-NBDG and then analyzed using a FCM. (D) ROS assay of SDHB-overexpressing cells. Each dot represents a sample. All data shown as mean ± SEM. *P<0.05; ***P<0.001. SDHB, succinate dehydrogenase enzyme B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OE, overexpression; ATP, adenosine triphosphate; ROS, reactive oxygen species; OLP, oral lichen planus; WB, western blot; FCM, flow cytometer; SEM, standard error of the mean.

SDHB is a potential therapeutic target for OLP patients

Additional NOM and OLP mucosa tissues were collected to verify changes in glycolysis and HIF-1α pathways. The WB analysis showed that PKM2, GLUT1, and MMP9 were up-regulated in OLP tissues compared with NOM, whereas HK2 expression was down-regulated (*Figure 6A,6B*). The same results were obtained at the mRNA level (*Figure 6C*).

Discussion

Our results provide a potential mechanism for the correlation between OLP development and mitochondrial dysfunction. In this study, several cellular models of OLP development were established *in vitro*, and SDHB expression was indeed significantly reduced with OLP occurrence, accompanied by a large amount of energy production. The stable reduction of SDHB led to cellular oxygen environmental changes and mitochondrial respiration impairment, thereby promoting the transition from aerobic respiration to glycolysis (36,37). In the process of cancer, the significant enhancement of glycolysis is the main means to provide energy for tumor proliferation and development (35). As Warburg described, compared to surrounding normal tissue, cancer has an increased reliance on the glycolytic pathway, and this is required to produce energy and synthesize macromolecules for sustaining the fast cellular growth with large amounts of oxygen (38).

According to our results, SDHB down-regulation within OLP cells and the change of cell biogenetics from aerobic respiration to glycolytic metabolism provide the plausible explanation for possible cellular deterioration and transformation to cancer. The SDHB low expression cells had nearly completely lost MMP and reduced ROS concentrations, indicating severe mitochondrial respiration disruption. Our findings also suggest that as SDHB decreases, cellular glucose uptake increases, changes in related biological genetic enzymes further provide a Warburg-like effect that has occurred in OLP as precancerous lesions. The overexpression of SDHB resulted in an unpredictable decrease in ATP production and $\Delta\psi m$, but increased ROS generation. Besides, cells overexpressing SDHB exhibited slightly decreased glucose absorption, suggesting that overexpression of SDHB induces some reduction in glycolytic activity. However, based on

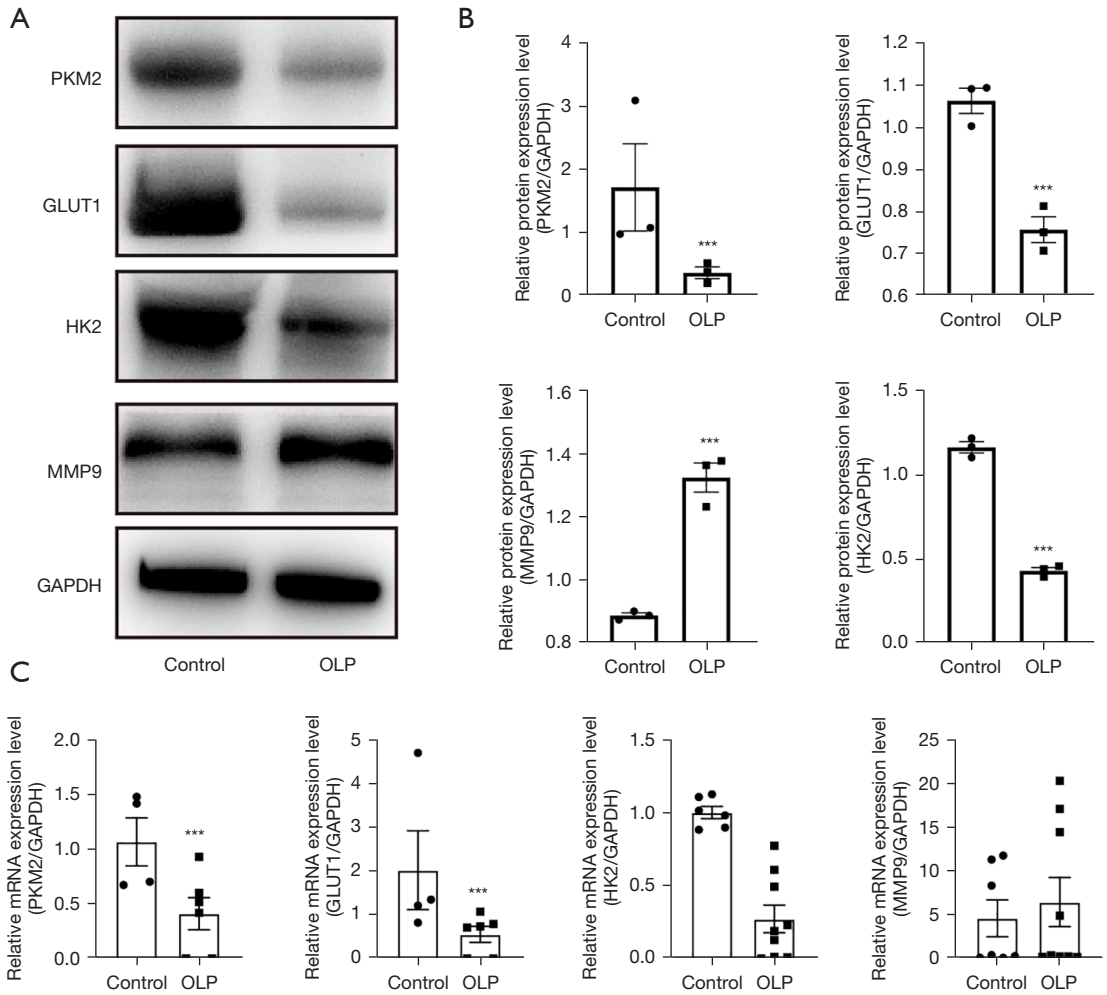

Figure 6 SDHB is a potential therapeutic target for OLP patients. (A,B) The protein levels of PKM2, GLUT1, HK2, and MMP9 in NOM and OLP tissues. (C) The PKM2, GLUT1, HK2, and MMP9 mRNA levels in NOM and OLP tissues. Each dot represents a sample. All data shown as mean ± SEM. ***P<0.001. PKM2, pyruvate kinase M2; GLUT1, glucose transporter type 1; HK, hexokinase; MMP9, matrix metallopeptidase 9; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OLP, oral lichen planus; mRNA, messenger RNA; NOM, normal oral mucosa; SEM, standard error of the mean.

careful analysis of the reduced levels of Δψm in SDHB overexpression or silencing cells, the reduced Δψm in cells with SDHB overexpression significantly decreased relative to cells with SDHB silencing. Therefore, SDHB up-regulation may have a mild impact on the damaging TCA cycle, later leading to glycolytic pathway dysregulation.

Although the exact mechanism by which SDH disruption in mitochondrial metabolism leads to tumorigenesis is unclear, it was recently reported that SDHB mutations could lead to dysfunction of the respiratory chain together with stabilization of HIFs, followed by the pseudohypoxic state (39). It may promote angiogenesis while facilitating tumorigenesis, but it does not explain all transformation of phenotypes, besides, its associated mechanisms remain to be elucidated. SDHB loss or reduction has a critical effect on the respiratory chain and TCA cycle, which causes bioenergetic shift to cytosolic glycolysis from mitochondrial respiration (32,34,37). Tumor cells depend on aerobic glycolysis to generate ATP, which is used as the antitumor therapeutic target. Inhibiting glycolysis can avoid ATP generation and reduce tumor cell proliferation. Therefore, attenuated glucose metabolism in cancer cells has been the

possible new area to develop the new antitumor treatments. Cases with SDHB down-regulation within precancerous lesions and dismal prognostic outcomes are considered for drug treatments targeting the Warburg effect (40,41). From a clinical perspective, our current findings suggest SDHB as an effective indicator of OLP-induced carcinogenesis and a possible therapeutic target.

Conclusions

We found that altered SDHB expression was closely associated with OLP progression. At cell level, decreased SDHB led to accumulation of succinate, which then promoted HIF-1α activation and impaired mitochondrial respiration. Our study suggests that SDHB reduction promotes OLP by impairing mitochondrial respiratory function.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5999/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5999/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5999/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Research Ethics Committee of Huashan Hospital, Fudan University (No. 2019-589) and informed consent was taken from all the patients.

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Supplementary

Table S1 The information of study participants

Patient ID	Gender	Age (years)	Stie of OLP lesions	Type of OLP lesions	Follow-up (mo)	Pathological number	Asociated condition	HE	Primary site of OSCC
HSKQ-1	F	57	BM	I	14	X20-03738	None	OLP	–
HSKQ-2	F	76	BM, LU	I	14	X20-03806	None	OLP	–
HSKQ-3	F	64	BM, T	I	6	X21-00849	Hypertension	OLP	–
HSKQ-4	M	37	BM, T	I	12	X20-06210	None	OLP	–
HSKQ-5	M	34	BM, T, LU, G	II	12	X20-05982	None	OLP	–
HSKQ-6	F	48	BM	I	9	X20-08874	None	OLP	–
HSKQ-7	M	29	BM	I	9	X20-08834	None	OLP	–
HSKQ-8	F	59	G, BM	I	7	X20-09842	Hypertension	OLP	–
HSKQ-9	F	42	BM	I	12	X20-06209	None	OLP	–
HSKQ-10	F	62	BM, G	I	9	X20-09557	None	OLP	–
HSKQ-11	F	62	BM	I	9	X20-09556	None	OLP	–
HSKQ-12	F	60	T, BM	I	15	X20-03190	None	OLP	–
HSKQ-13	M	53	BM	I	27	X19-02823	Hypertension	OLP	–
HSKQ-14	F	56	T, BM	II	27	X19-02725	None	OLP	–
HSKQ-15	F	55	BM, T	I	15	X20-02697	None	OLP	–
HSKQ-16	M	50	T, BM	I	12	X20-05932	None	OLP	–
HSKQ-17	M	27	LU, BM	I	10	X20-07583	None	OLP	–
HSKQ-18	M	24	BM	I	11	X20-07313	None	OLP	–
HSKQ-19	F	69	BM	I	13	X20-05677	None	OLP	–
HSKQ-20	M	52	BM	II	13	X20-05423	None	OLP	–
HSKQ-21	M	52	BM	I	14	X20-03863	None	OLP	–
HSKQ-22	M	68	BM	I	13	X20-04951	None	OLP	–
HSKQ-23	M	45	BM	I	12	X20-04968	None	OLP	–

OLP, oral lichen planus; HE, hematoxylin-eosin; OSCC, oral squamous cell carcinoma; BM, buccal mucosa; LU, under of lip; T, tongue; G, gingiva.

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