Research Article

Overexpression of PTPRZ1 Regulates p120/β-Catenin Phosphorylation to Promote Carcinogenesis of Oral Submucous Fibrosis

Liwei Ma,1,2,3,4 Ting Shen,5,6 Hui Peng,1,4 Jianjun Wu,2 Wenjin Wang,2 and Xing Gao2,3,4,6,7

1Department of Oral Medicine, Center of Stomatology, Xiangya Hospital, Central South University, Changsha, Hunan Province 410008, China
2Department of Oral and Maxillofacial Surgery, Center of Stomatology, Xiangya Hospital, Central South University, Changsha, Hunan Province 410008, China
3Center of Oral and Maxillofacial Cancer (COMAC), Xiangya Hospital, Central South University, Changsha, Hunan Province 410008, China
4Institute of Oral Cancer and Precancerous Lesions, Central South University, Changsha, Hunan Province 410008, China
5Xiangya Stomatological Hospital, Xiangya School of Stomatology, Central South University, Changsha, Hunan Province 410008, China
6Key Laboratory of Molecular Radiation Oncology Hunan Province, Changsha, Hunan Province 410008, China
7National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China

Correspondence should be addressed to Xing Gao; 45210700@qq.com

Received 22 July 2021; Revised 22 December 2021; Accepted 3 January 2022; Published 23 February 2022

Academic Editor: Dali Zheng

Copyright © 2022 Liwei Ma et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Oral submucous fibrosis (OSF) is a potentially malignant disease of the oral cavity. New molecular predictors are needed to identify the high risk of malignant transformation in potentially malignant oral lesions. Our purpose is to explore PTPRZ1 and p120/β-catenin pathogenesis in the carcinogenesis of OSF to identify novel drug targets.

Methods. kfl_he expression of PTPRZ1, p120, and β-catenin in clinical tissues was detected. kfl_hen, PTPRZ1, p120, and β-catenin, RhoA, Rac1, CDC42, cyclin D1, and c-myc expressions were detected by qRT-PCR and western blot. CCK-8 was applied to measure hOMF cells viability. Wound healing and transwell assay were applied to measure cell migration and invasion. Western blot and IF detected the distribution of p-p120 and p-β-catenin. Tumor formation experiment explored PTPRZ1 effects on OSF. Results. PTPRZ1, p120, and β-catenin were abnormally expressed in cancer tissues. PTPRZ1 regulated the phosphorylation of p120/β-catenin. Western blot and IF showed that in the oe-NC group, p-p120 and p-β-catenin were expressed in the cell membrane. p-p120 and p-β-catenin were expressed in the cytoplasm and nucleus of the oe-PTPRZ1 group. In vitro experimental results revealed overexpression of PTPRZ1 and β-catenin, and silencing of p120 promoted cell proliferation, migration, and invasion. The tumor volume and weight in the sh-PTPRZ1 group were significantly reduced. IHC revealed the positive rate of PTPRZ1 was also low.

Conclusions. Overexpression of PTPRZ1 regulated the phosphorylation of p120/β-catenin to promote OSF malignancy.

1. Introduction

Oral submucous fibrosis (OSF) is a chronic, insidious, and progressive oral mucosal disease [1, 2]. It is characterized by abnormal collagen deposition, which can produce scars and tissue fibrosis [3]. OSF is a common oral precancerous lesion in Asian countries, especially in areas where the culture of areca nut chewing is prevalent. The age of illness is between 20 and 40 years old [4]. The patient has submucosal fibrosis, ulcers, dry mouth, burning sensation, and restricted mouth opening, which greatly affects the quality of life of the patient [3]. Among them, about 1.5%–15% of all cases are transformed into malignant tumors [5]. It was reported that the incidence of oral squamous cell carcinoma (OSCC) caused
by chewing areca is high in Asia-Pacific countries [6]. In order to reduce OSF occurrence, early detection of pre-malignant diseases, understanding the mechanism of OSF induction and transformation, and taking active therapeutic interventions are very important. Although many OSF lesions biomarkers have been identified and different drug combinations trials have been carried out, clinicians still adopt conservative treatments, mainly focusing on alleviating OSF symptoms. Therefore, new molecular predictors are needed to identify the high risk of malignant transformation in potentially malignant oral lesions.

Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1) is a member of the RPTP family [7]. PTPRZ1 has different functions in the occurrence, development, and metastasis of cancer, which further affects cancer treatment and prognosis. Studies have found that PTPRZ1 is abnormally expressed in various types of tumors. PTPRZ1 is upregulated in human astrocytomas, cervical carcinoma, and small-cell lung carcinoma [8–10]. It is downregulated in breast invasive carcinoma and stomach adenocarcinoma [11]. Some studies also have shown that PTPRZ1 plays a vital role in the occurrence, development, invasion, and metastasis of OSF carcinogenesis and can be used as a molecular marker for early diagnosis and a target gene for treatment [12]. It has been confirmed that PTPRZ1/β-catenin is an important pathway that regulates tumorigenesis. PTPRZ1 can enhance β-catenin protein expression in the nucleus of human renal cell carcinoma and participates in regulating proliferation by activating β-catenin and its downstream genes [13].

Cathepsin cell-cell adhesion complex plays a vital role in the development and progression of cancer [14]. p120-catenin (p120) is a cytoplasmic molecule closely related to E-cadherin. P120 coexists with β-catenin or γ-catenin in the E-cadherin complex and plays a vital role in stabilizing the E-cadherin-catenin complex [15]. The expression of p120 has been extensively studied in many human cancers. Studies have shown that the expression of phosphorylated p120-catenin has predictive value for the progression of oral cancer [16]. In addition, phosphorylation of p120 is an effective substrate of the oncopgenic Src family, and its phosphorylation is related to cell transformation [17]. β-Catenin is a protein containing a central armadillo (ARM) domain (residues 140–664), amino-terminus domain (NTD), and carboxyl terminus domain (CTD) [18]. It is encoded by the CTNNB1 gene and is a dual-function protein that regulates the coordination of cell-cell adhesion and gene transcription [19, 20]. Abnormal expression of β-catenin is related to metastatic oral squamous cell carcinoma (OSCC) with positive cervical lymph nodes [21]. However, little is known about the relationship between OSF and p120/β-catenin.

Therefore, we hypothesized that the PTPRZ1/p120/β-catenin axis might play a role in the malignant transformation of OSF. We verified that OSF malignant transformation is related to the PTPRZ1/p120/β-catenin axis in human tissues. In addition, we used arecoline-induced cell models to elucidate the mechanism of PTPRZ1 regulated the phosphorylation of p120/β-catenin to promote OSF malignant transformation and to clarify its value as a potential therapeutic target, so as to provide novel ideas and theoretical evidence for the prevention and treatment of OSF malignant transformation.

2. Materials and Methods

2.1. Clinical Tissue Samples. Forty participants were recruited from Xiangya Hospital of Central South University. Human normal oral mucosal tissue, early OSF tissue, middle OSF tissue, and OSCC tissue were collected. The groups were normal group, early-OSF group, medium-OSF group, and the OSCC group. We have obtained the written informed consent of the subjects before the start of the study and obtained the approval of the Medical Ethics Committee of Xiangya Hospital (No. 201703635).

2.2. Cell Culture and Treatment. Normal oral epithelial cells (HNOEC cells), OSCC cells (CAL27 cells), and human oral mucosa fibroblasts (hOMF cells) were purchased from the Cell Bank of the Chinese Academy of Sciences. HNOEC cells were cultured in a Minimum Essential Medium containing 10% FBS (Thermo Fisher Scientific). CAL27 cells were cultured in DMEM containing 10% FBS and 1% penicillin/streptomycin (P/S, C0222, Beyotime Biotechnology). hOMF cells were cultured in 90% H-DMEM and 10% FBS containing 100 U/mL P/S medium. They were placed at 37°C, cultivated in a humidity chamber with 5% CO2. They were divided into the control (HNOEC cells), OSCC (CAL27 cells), OSF (hOMF cells), OSF+arecoline (hOMF cells), oe-NC (hOMF cells), and oe-PTPRZ1 (hOMF cells) groups. The OSF + arecoline group was treated with 0.2 μg/mL arecoline for 24 h in hOMF cells [22]. In order to overexpress PTPRZ1, the PTPRZ1 sequence was linked to the LV003 vector. According to the instructions, the PTPRZ1-vector plasmid was transfected into hOMF cells using lipofectamine 3000 reagent. The oe-NC group was transfected with oe-NC plasmid in hOMF cells, and the oe-PTPRZ1 group was transfected with PTPRZ1-vector plasmid in hOMF cells. The shRNA-1 targeting human p120 (5′-GATAGAAGCTGAGCTGAAGTTGAA-3′), shRNA-2 targeting human p120 (5′-GGATGCTGTTAGACCTTCTACTTGA-3′), and shRNA-3 targeting human p120 (5′-CGATCCTAACCATTGAGCTGATAA-3′) synthesized by Sangon Biotech (Shanghai, China) and the corresponding negative control sh-NC (5′-UCUCGGGAAAGUGUCAGUTT-3′) were used to knock down the expression of p120. They were divided into the sh-NC, sh-p120-1, sh-p120-2, and sh-p120-3 groups. In order to overexpress β-catenin, the β-catenin sequence was linked to the vector LV003. According to the instructions, β-catenin-vector plasmid was transfected into hOMF cells using lipofectamine 3000 reagent. They were divided into the oe-NC group and the oe-β-catenin group.
2.3. Quantitative Real-Time PCR (qRT-PCR). QRT-PCR was performed to detect PTPRZ1, p120, β-catenin, RhoA, Rac1, CDC42, cyclin D1, and c-myc expression levels. To put it simply, total RNA was extracted by the Trizol method. RNA was reversely transcribed into cDNAs in accordance with the instruction of a reverse transcription kit (CW2569, CWBIO, China). SYBR Green qPCR mix (Invitrogen) was performed to test genes relative expression in ABI 7900 system. The relative level of the gene was calculated by the $2^{-ΔΔCt}$ method with GAPDH as the internal gene. The primer sequence used in this study is shown in Table 1. Each group was tested three times.

2.4. Western Blot (WB). RIPA lysis buffer (#P0013 B, Beyotime Biotechnology) was applied to extract the total protein from cells and tissues according to the instructions. The protein of each group was quantified according to the BCA protein determination kit, and the SDS-PAGE loading buffer (#MB2479, Meilunbio) was mixed; the mixture was heated for 5 minutes in boiling water at 100°C. The protein was adsorbed on the PVDF membrane by gel electrophoresis, and then it was sealed at 2 hours with a 5% skim milk solution at room temperature. PTPRZ1 (55125-1-AP, 1:1,000, Proteintech), p120 (12180-1-AP, 1:3,000, Proteintech), p-p120 (ab173578, 1:200, Abcam), primary antibodies were incubated for 90 minutes at room temperature. After ECL exposure, it was placed in xylene for 10 min and then sealed with neutral gum and observed under a microscope. Each group was tested three times.

2.5. Immunohistochemistry (IHC). The expression of PTPRZ1, p-p120, and β-catenin was detected by IHC in human and mouse tumor tissues. The slices were roasted at β-Actin-R AGCACAGCCTGGATAGCAAC
β-Actin-F ACCCTGAAGTACCCCATCGAG
Cyclin D1-F ACCTCTTCATTATCTCATGGCT
Cyclin D1-R TGCCITCCGACCTGCTGAC
Rac1-F AACATCGTCAACTACAGAC
Rac1-R AACATCGTCAACTACAGAC
CDC42-F ACAACACAGAAGCTATCACTCC
CDC42-R CTGGCGGCCTTCTTCCGGTC
C-myc-F ACACTAAATCCCAGCTCTC
C-myc-R CGGACCTTGTGTCTCTGTAGT
β-Actin-F ACCCTGAATACCCCATCGAG
β-Actin-R AGCAGACCTGGATACGACAC

removal, it was placed in xylene for 10 min and then sealed with neutral gum and observed under a microscope. Each group was tested three times.

2.6. Immunofluorescence (IF). IF detected the distribution of p-p120 and p-β-catenin. The slides were removed and washed with PBS 2–3 times. Then slides were fixed for 4% paraformaldehyde for 30 min and PBS was washed 3 times for 5 minutes. Then, it was permeabilized with 0.5% TritonX-100 at 37°C for 30 min. After washing with PBS, 5% BSA was sealed at 37°C for 1 h, p-p120 (ab173578, 1:50, Abcam) and p-β-catenin (ab27798, 1:50, Abcam) were incubated overnight at 4°C. PBS was washed 3 times for 5 minutes. Diluted CoraLite594-conjugated Goat Anti-Rabbit IgG (SA00013-4, 1:200, Proteintech) was added. Then they were incubated at 37°C for 90 min, and PBS was washed 3 times for 5 minutes. Then they were dyed with DAPI (Wellbio) at 37°C for 10 min. The plates were sealed and observed under a fluorescence microscope. Each group was tested three times.

2.7. Cell Counting Kit 8 (CCK-8) Assay. The cells in the logarithmic growth phase were seeded in 96-well plates, and each well was seeded with $1 \times 10^4$ cells. Then, the cells were cultured in a 37°C, 5% CO₂ incubator for 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h, respectively. After experimental treatment, 10 μL CCK-8 reagent was added in an incubator for 2 h. The microplate reader (Infinite M200, Tecan, Austria) was performed to measure absorbance at 450 nm to assess cell viability in each group. Each group was tested three times.

2.8. Wound Healing. When the cells grew at a fusion rate of 90%, the cells were washed once with sterile PBS, scratched with a pipette tip, and then washed once with PBS. Then we removed the scratched cells and added serum-free DMEM

Table 1: The primers used in this study.

| Primer ID | Primer sequence |
|-----------|-----------------|
| PTPRZ1-F  | TGCAGAGGCTCTGATGTTGACCTT |
| PTPRZ1-R  | TGGCATCTCGTTCTCCAGACAG |
| p120-F    | ATGAGTGGTTCTCCAGAGGA  |
| p120-R    | GCAGACGAGGCGGATGTAT  |
| β-Catenin-F | ATCTTCTGATTTGCAAAGCATC |
| β-Catenin-R | AGCAGACGATAGACCTT |
| RhoA-F    | ACACACCGAGCGCTATTCA |
| RhoA-R    | CCAAGACGATAGACCTT |
| Cyclin D1-F | ACCTCTTCATTATCTCATGGCT |
| Cyclin D1-R | TGCACCTCCGACCTGCTGAC |
| Rac1-F    | AACATCGTCAACTACAGAC |
| Rac1-R    | AACATCGTCAACTACAGAC |
| CDC42-F   | ACAACACAGAAGCTATCACTCC |
| CDC42-R   | CTGGCGGCCTTCTTCCGGTC |
| c-myc-F   | ACACTAAATCCCAGCTCTG |
| c-myc-R   | CGACTCTTGTCTCTGTAGT |
| β-Actin-F | ACCCTGAATACCCCATCGAG |
| β-Actin-R | AGCAGACCTGGATACGACAC |
high-sugar medium. Photographs were taken at 0 h and 24 h to detect cell migration. Each group was tested three times.

2.9. Transwell Assay. The cells were collected using the transwell system, resuspended with serum-free DMEM, and inoculated into transwell plate lumen. The inner side of the chamber on the transwell plate was precoated with Matrigel basement membrane matrix (BD Biocoat). The Matrigel glue was dissolved overnight at 4°C and then diluted with pre-cooled basal medium (Matrigel: medium) at the ratio of 1:3. 40 μL of Matrigel glue was added to precooled transwell chamber and incubated at 37°C for 2 h to make Matrigel glue solidify. Cells were counted with basal medium and adjusted to 1 × 10⁶/mL, added 100 μL to the upper chamber of the transwell chamber and 600 μL of complete medium to the lower chamber. After incubating at 37°C for 24 h, the cells on the surface of the upper chamber were wiped with wet cotton swabs, fixed with 4% paraformaldehyde for 20 min, stained with 0.5% crystal violet for 5–10 min, and observed and photographed under a microscope. Each group was tested three times.

2.10. Cell Cycle Assay. The cell suspension was collected and centrifuged to obtain cell precipitate. PBS was washed 2–3 times to prepare single-cell suspension, and cells number was adjusted to 1 × 10⁶ cells/mL. A total of 150 μL propidium iodide (PI) was added and stained at 4°C for 30 min in dark. PI was excited by a 488 nm argon-ion laser and received by a 630 nm pass filter. Ten thousand cells were collected through FSC/SSC scatter plot. The gating technique was used to eliminate adhesion cells and fragments, and the percentage of each cell cycle on the fluorescence histogram of PI was analyzed. Each group was tested three times.

2.11. Apoptosis Assay. The treated cells were digested and collected with trypsin without EDTA. Cells were washed with PBS twice and centrifuged at 2,000 rpm for 5 min each time; about 5 × 10⁵ cells were collected. We added 500 μL of binding buffer to suspend cells. After adding 5 μL Annexin V-FITC (KGA108, KeyGen) and mixing well, 5 μL propidium iodide (PI) was added. The reaction time was 15 min at room temperature and kept away from light. Flow cytometry (A00-1-1102, Beckman) was performed within 1 h. Each group was tested three times.

2.12. EDU. According to the EDU detection kit (RiboBio), the proliferation rate of cells was detected. First, the EDU was marked. The EDU solution was diluted with cell medium at the ratio of 1:1,000:1 to prepare an appropriate amount of 50 μm EDU culture medium. The cells were incubated overnight in 100 μL 50 μM EDU medium, and PBS was washed 2 times for 5 minutes. Then 50 μL cell fixation solution (4% paraformaldehyde) was added, and it was incubated for 30 min at room temperature. After adding 50 μL 2 mg/mL glycine and incubating in a decolorizing shaker for 5 min, PBS was washed for 5 minutes. Next, 100 μL penetrant was added to decolorize, and it was incubated in a decolorizing shaker for 10 minutes. PBS was washed once for 5 minutes. We added 100 μL of 1× Apollo® staining reaction solution, and it was incubated for 30 minutes in a decolorizing shaker at room temperature and kept away from light. Then we added 100 μL of penetrant to decolorize and washed 2–3 times on decolorizing shaker for 10 minutes each time. After adding 100 μL methanol to wash 1–2 times for 5 minutes each time and washing once with PBS for 5 minutes, DNA was stained and observed immediately after staining. Each group was tested three times.

2.13. In Vivo Tumorigenesis. Twelve SPF-grade, 4-week-old mice were randomly divided into OSCC and sh-PTPRZ1 group with 6 mice in each group. Animal studies were approved by the Medical Ethics Committee of Xiangya Hospital (No. 20173637). The short hairpin targeting PTPRZ1 (sh-PTPRZ1) synthesized by Sangon Biotech and corresponding negative control sh-NC were used to knock down PTPRZ1 expression. The OSCC group was injected with sh-NC treated OSCC cells, and the sh-PTPRZ1 group was injected with sh-PTPRZ1 treated OSCC cells, and they were injected into nude mice axilla [23]. The tumor volume of each group was measured at 4, 7, 11, 14, 17, 21, and 24 d. The nude mice were sacrificed at 24 days, and the tumor mass of each group was detected. The positive rate of PTPRZ1 in tumor tissues was detected by IHC.

2.14. Statistical Analysis. Graphpad 8.0 was applied for statistical analysis, and experimental data were expressed as mean ± SD, which was repeated at least 3 times. Pearson correlation coefficient analyzed the correlation between PTPRZ1 and p120. One-way ANOVA was used for comparison between the two groups. p < 0.05 was considered statistically significant.

3. Results

3.1. Expression of PTPRZ1, p120, and β-Catenin in Tissues. To investigate the expression of PTPRZ1, p120, and β-catenin in OSF tissues and OSCC tissues, qRT-PCR was first performed. The results revealed the expression of PTPRZ1 and β-catenin in early-OSF, middle-OSF, and OSCC groups increased compared with the normal group, while the expression of p120 decreased (Figure 1(a)). Western blot results revealed PTPRZ1, and p-p120/p120 expressions increased in the early-OSF, middle-OSF, and OSCC groups, while p-β-catenin/β-catenin decreased (Figure 1(b)). Pearson correlation coefficient analysis showed a positive correlation between PTPRZ1 and β-catenin and a negative correlation between PTPRZ1 and p120 (Figure 1(c)). The results of IHC revealed the positive rate of PTPRZ1, p-p120, and p-β-catenin in early-OSF, middle-OSF, and OSCC groups increased compared with the normal group, and the positive rate of p-β-catenin decreased (Figure 1(d)). Besides, p-β-catenin was mainly expressed in the cell membrane, but hardly in the cytoplasm and nucleus. PTPRZ1 and p-P120 were detected in all parts of the cells. The above results
### Relative expression of PTPRZ1, p120, and β-catenin

| Condition | PTPRZ1 | p120 | p-p120 | β-catenin | p-β-catenin | β-actin |
|-----------|--------|------|--------|-----------|-------------|--------|
| Normal    | 0.5    | 1.0  | 1.5    | 0.5       | 1.0         | 1.0    |
| Early-OSF | 1.0    | 1.5  | 1.0    | 1.0       | 0.5         | 0.5    |
| Medium-OSF| 2.0    | 2.0  | 2.0    | 2.0       | 2.0         | 2.0    |
| OSCC      | 3.0    | 3.0  | 3.0    | 3.0       | 3.0         | 3.0    |

### Protein expression

| Condition | PTPRZ1 | p120 | p-p120 | β-catenin | p-β-catenin | β-actin |
|-----------|--------|------|--------|-----------|-------------|--------|
| Normal    | 0.5    | 1.0  | 1.5    | 0.5       | 1.0         | 1.0    |
| Early-OSF | 1.0    | 1.5  | 1.0    | 1.0       | 0.5         | 0.5    |
| Medium-OSF| 2.0    | 2.0  | 2.0    | 2.0       | 2.0         | 2.0    |
| OSCC      | 3.0    | 3.0  | 3.0    | 3.0       | 3.0         | 3.0    |

### Correlation

- PTPRZ1 vs. β-catenin: $R = -0.8777$, $P < 0.0001$
- PTPRZ1 vs. p120: $R = -0.8777$, $P < 0.0001$

**Figure 1:** Continued.
PTPRZ1, p120, and β-catenin are abnormally expressed in cancer tissues.  

### 3.2 Overexpression of PTPRZ1 Regulated the Phosphorylation of p120/β-Catenin

P120/β-catenin has been shown to affect the development of OSCC [14]. Next, we wanted to investigate whether PTPRZ1 affects OSF carcinogenesis by p120/β-catenin. PTPRZ1 mRNA and protein expressions in the OSCC group (CAL27 cells) were increased compared with the control group (HNOEC cells). Compared with the OSF group (hOMF cells), PTPRZ1 mRNA and protein expressions were increased in the OSF+arecoline group (Figures 2(a) and 2(b)). As shown in Figures 2(c)–2(g), PTPRZ1, p-p120, and p-β-catenin mRNA and protein expressions in the OSF+arecoline group were increased compared with the OSF group (hOMF cells), while the expressions of p-β-catenin and p120 were decreased. Compared with the oe-NC group (hOMF cells), PTPRZ1, p-p120, and β-catenin mRNA and protein levels were increased, and p-β-catenin and p120 expressions were decreased in the oe-PTPRZ1 (hOMF cells). At the same time, the content of p-P120 in the cytoplasm was higher than that in the nucleus, but the change trend in both was consistent. IF showed that p-p120 and p-β-catenin were expressed in the cell membrane in the oe-NC group (hOMF cells). In the oe-PTPRZ1 (hOMF cells), p-p120 and p-β-catenin were expressed in cytoplasm and nucleus (Figure 2(h)). These results revealed overexpression of PTPRZ1 regulated p120/β-catenin phosphorylation.  

### 3.3 Overexpression of PTPRZ1 Promoted Oral Cell Carcinogenesis

From the above experiments, it was concluded that PTPRZ1 could regulate the expression of p120 and β-catenin. Then we want to further explore whether PTPRZ1 can promote OSF malignancy. As shown in Figure 3(a), compared with the OSF group (hOMF cells), the viability of the cells of the OSF+arecoline group (hOMF cells) after 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h also increased. At the same time, the activity of the cells after 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h of the oe-PTPRZ1 group (hOMF cells) were also increased compared with the oe-NC group (hOMF cells). Besides, compared with the OSF group (hOMF cells), the cells migration and invasion ability of the OSF+arecoline group (hOMF cells) also increased. The cells migration and invasion ability of the oe-PTPRZ1 group (hOMF cells) were also increased compared with the oe-NC group (hOMF cells; Figures 3(b)–3(f)). Compared with the OSF group (hOMF cells), the G0/G1 phase also decreased; the S phase increased; and apoptosis decreased in the oe-NC group (hOMF cells; Figures 3(g)–3(i)). These confirmed our conjecture that overexpression of PTPRZ1 promoted the carcinogenesis of OSF.  

### 3.4 Knockdown of p120 Pathway Promoted Oral Cell Carcinogenesis

Next, we explored the effect of overexpression of p120 on OSF. Studies have shown that p120...
Figure 2: Continued.
aggregated to affect RhoA, Rac1, and CDC42 [24]. After we tried to treat hOMF cells with three kinds of sh-p120, the sh-p120-2 group with the most significant knockout effect was selected for follow-up study (Figure 4(a)), which was called the “sh-p120” group. As shown in Figure 4(b), the cell viability of the sh-p120 group (hOMF cells) increased compared with the sh-NC group (hOMF cells) after 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h. As shown in Figures 4(c) and 4(d), p-p120, RhoA, Rac1, and CDC42 expressions in the sh-p120 group (hOMF cells) increased compared with the sh-NC group (hOMF cells), while p120 decreased. Compared with the sh-NC group (hOMF cells), the cell proliferation in the sh-p120 group (hOMF cells) increased; the S phase increased; the G0/G1 phase decreased; and the apoptosis decreased (Figures 4(e)–4(g)), indicating that the knockdown p120 promoted OSF cell carcinogenesis.

3.5. Activated β-Catenin Pathway Promoted Oral Cell Carcinogenesis. Then, we want to explore the effect of the expression of β-catenin pathway on OSF. Studies have shown that cyclin D1 and c-myc are involved in the occurrence, development, and promotion of cancer [25]. As shown in Figure 5(a), the cell viability of the oe-β-catenin group (hOMF cells) increased compared with the oe-NC group (hOMF cells) after 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h. As shown in Figures 5(b) and 5(c), qRT-PCR and western blot detection results revealed compared with the oe-NC group (hOMF cells), cyclin D1, c-myc, and β-catenin increased in the oe-β-catenin group, while p-β-catenin expression decreased. Compared with the oe-NC group (hOMF cells), the cell proliferation in oe-β-catenin group increased; the S phase increased; the G0/G1 phase decreased; and the apoptosis decreased (Figure 5(d)–5(f)). It showed that activated β-catenin promoted OSF cell carcinogenesis.

3.6. Silencing PTPRZ1 Inhibited the Development of OSCC. In order to further investigate PTPRZ1 effects on OSCC, we performed nude mice tumorigenesis experiment and injected the OSCC cells treated with sh-PTPRZ1. As shown in Figures 6(c) and 6(d), the tumor volume in the sh-PTPRZ1 group was significantly reduced compared with the OSCC group. As shown in Figures 6(c) and 6(d), tumor weight was significantly reduced, and the positive rate of PTPRZ1 was lower. These results suggested that silencing PTPRZ1 inhibited the development of OSCC.
Figure 3: Continued.
**Figure 3: Continued.**
4. Discussion

OSF is a potentially malignant disease of the oral cavity. At present, conservative treatment to alleviate OSF is the main choice of clinical treatment, and there is a lack of good treatment methods for OSF. In this paper, we studied the possible role of the PTPRZ1/p120/β-catenin axis in the malignant transformation of OSF through clinical samples, arecoline-treated cell models, and nude mice tumorigenesis experiment. We found that the OSF malignant transformation in human tissues is related to the PTPRZ1/p120/β-catenin axis. In addition, PTPRZ1 could regulate the phosphorylation of the p120/β-catenin pathway to promote OSF malignancy.

PTPRZ1 is a transmembrane protein tyrosine phosphatase. Its expression is different in different types of cancer. In view of the up- and downregulation of PTPRZ1 in different cancers, the pharmacological inhibition or activation of PTPRZ1 may be a promising strategy for tumors treatment. PTPRZ1 plays a role in cell proliferation, adhesion and migration, EMT, cancer stem cells, and treatment resistance by interacting or binding with several molecules [11]. It has been reported that PTN is one of the most important ligands of PTPRZ1. The interaction between PTPRZ1 and PTN increases the steady-state tyrosine dephosphorylation of β-catenin [26]. p120 is an adhesion junction protein, which plays a role in the adhesion and signal transduction between cells [27]. Attenuation of p120 expression may be a potential treatment strategy for pulmonary fibrosis [28]. According to the TCGA data, PTPRZ1 may be related to OS and DFS in various cancers, which suggests PTPRZ1 is involved in survival signals [29]. PTPRZ1 has an important impact on the survival of OSCC patients, and the risk of death within 5 years of positive PTPRZ1 patients is 8 times lower than that of patients with negative PTPRZ1 [30]. Knockdown of P120 promotes OSCC cell proliferation and tumor growth through C-y1 signal transduction [31]. p-p120 promotes the progression and invasion of OSCC cells and may be a potential marker of OSCC disease [16]. As for the decrease of P120 and the increase of p-p120 expression level, this may be similar to the changes after β-catenin phosphorylation. Phosphorylation of β-catenin initiates degradation of the total β-catenin protein, resulting in a decrease in total protein content [32, 33]. In this paper, we found PTPRZ1, expression in OSF, and OSCC tissue was higher than that of normal oral mucosa tissue group, while p120 expression was lower. Besides, p-β-catenin was mainly expressed on the cell membrane, and PTPRZ1 and P–P120 were detected in all parts of cells with the same change trend. Pearson correlation coefficient analysis showed that PTPRZ1 was positively correlated with β-catenin and negatively correlated with p120. We found that the OSF malignant transformation is related to the PTPRZ1/p120/β-catenin axis.

Studies have shown that chronic oxidative stress leads to the amplification and overexpression of PTPRZ1 protein tyrosine phosphatase, thereby activating the β-catenin pathway [34]. In the rat renal cell carcinoma model, β-catenin pathway regulated by PTPRZ1 was the mediator of rat renal cell carcinoma carcinogenesis. β-catenin translocated to the nucleus and activated the downstream target genes [13]. PTPRZ1 promoted β-catenin tyrosine dephosphorylation and further increased the involvement of β-catenin in T cell factor (TCF) mediated transcription [35], p120 was an upstream regulator of neurogenesis and cell cycle pathways in glioma patients and a predictor of poor clinical prognosis [36], p120 could inhibit RhoA and stabilize the cadherin and β-catenin on the plasma membrane [37], p120 stabilized the membrane association of β-catenin, thereby preventing the accumulation of β-catenin nuclei and the excessive activation of the WNT pathway during EMT [38]. We found that p-β-catenin was mainly expressed on the cell membrane, and PTPRZ1 and P–P120 were detected in all parts of cells with the same change trend. We found that PTPRZ1 could affect OSF carcinogenesis by regulating the phosphorylation of p120/β-catenin.

It was reported that β-catenin tyrosine phosphorylation led to a loss of intercellular adhesion, which in turn led to elevated cytoplasmic β-catenin levels, migrated to nucleus, and bound to transcription factor TCF/LEF. These led to
increased MYC and cyclin D1 expressions, stimulated cell cycle, and proliferation [39]. PTPRZ1 knockdown could reduce the number of nuclear β-catenin and inhibit cell proliferation, as well as reduce target genes cyclin D1 and c-myc expressions in renal cancer [34]. Our results revealed that cyclin D1 and c-myc expressions were increased after...
overexpression of β-catenin. P120 and its phosphorylation of Tyr228 could inhibit the proliferation and invasion of colon adenocarcinoma cells [40]. Previous studies reported that increased expression of p120 isoforms 1A not only can upregulate E-cadherin and β-catenin but also can downregulate Rac1 activity and inhibit cell invasion. On the contrary, overexpression of p120 isoforms 3A could cause CDC42 inactivation and RhoA activation and has a small effect on invasion [41]. It has also been reported that deletion of p120 can inactivate RhoA but increase the activity of CDC42 and Rac1 and promote the proliferation and invasion of lung cancer cells [42]. We found that RhoA, Rac1, and CDC42 expressions increased after silencing p120. Through a series of cell function experiments, we found that PTPRZ1 overexpression promoted cell proliferation, migration, and invasion. Silencing p120 and overexpression of β-catenin can also promote cell proliferation, migration, and invasion. In nude mice tumor formation experiments, it was found that silencing PTPRZ1 can inhibit the development of OSCC. These showed that PTPRZ1/p120/β-catenin was related to the development process of OSF.

Figure 5: Activated β-catenin pathway promoted oral cell carcinogenesis. (a) CCK-8 detected the proliferation rate of cells in each group at 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h (n = 3). (b) qRT-PCR was used to detect β-catenin, cyclin D1, and c-myc expressions (n = 3). (c) Western blot was used to detect β-catenin, p-β-catenin, cyclin D1, and c-myc protein expression levels (n = 3). (d) EDU detected the proliferation rate of cells in each group (n = 3). (e) Flow cytometry was performed to detect cell cycle (n = 3). (f) Annexin V-FITC/PI flow cytometry was used to detect the apoptosis rate of cells (n = 3). * p < 0.05 vs. the oe-NC group (hOMF cells); scale bar = 100 μm; and the magnification is 100 times.
5. Conclusions

Our results showed that overexpression of PTPRZ1 could regulate p120/β-catenin phosphorylation to promote the malignant transformation of OSF. Our research provided ideas for the pathogenesis of OSF carcinogenesis. It was clear that the PTPRZ1/p120/β-catenin axis might serve as a potential therapeutic target and will help enrich new therapeutic strategies for OSF.

Data Availability

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

Disclosure

Present address of Jianjun Wu is Hunan Key Laboratory of Oral Health Research & Hunan 3D Printing Engineering Research Center of Oral Care & Hunan Clinical Research Center of Oral Major Diseases and Oral Health & Xiangya Stomatological Hospital & Xiangya School of Stomatology, Central South University, Changsha, Hunan Province 410008, China. Present address of Wenjin Wang is Guangdong Provincial Key Laboratory of Stomatolgy, Hospital of Stomatolgy, Sun Yat-sen University, Guangzhou, Guangdong Province 510055, China.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant nos. 81703069 and 81700989), Fellowship of China Postdoctoral Science Foundation (grant nos. 2020M672523 and 2021T140747), and Natural Science Foundation of Hunan Province (grant no. 2021JJ31061).

References

[1] Q. Peng, H. Li, J. Chen, Y. Wang, and Z. Tang, “Oral submucous fibrosis in Asian countries,” Journal of Oral Pathology & Medicine, vol. 49, no. 4, pp. 294–304, 2020.
[2] N. Li, X. Jian, Y. Hu, C. Xu, Z. Yao, and X. Zhong, “Discovery of novel biomarkers in oral submucous fibrosis by microarray analysis,” Cancer Epidemiology Biomarkers & Prevention, vol. 17, no. 9, pp. 2249–2259, 2008.
[3] Y. W. Shen, Y. H Shih, L. J Fuh, and T. M Shieh, “Oral submucous fibrosis: a review on etiopathogenesis, diagnosis,
and therapy,” *International Journal of Molecular Sciences*, vol. 20, no. 12, 2019.

[5] Y.-Y. Wang, Y.-H. Tail, W.-C. Wang et al., “Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders,” *BMC Oral Health*, vol. 14, no. 1, p. 99, 2014.

[6] J. Wang, C. Jiang, N. Li et al., “The circEPSTI1/mir-942-5p/LTB2 axis regulates the progression of OSCC in the background of OSF via EMT and the PT3K/Akt/mTOR pathway,” *Cell Death & Disease*, vol. 11, no. 8, p. 682, 2020.

[7] K. Shitara, H. Yamada, K. Watanabe, M. Shimokawa, and Y. Yamaguchi, “Brain-specific receptor-type protein-tyrosine phosphatase beta is a chondroitin sulfate proteoglycan in vivo,” *Journal of Biological Chemistry*, vol. 269, no. 31, pp. 20189–20193, 1994.

[8] U. Ulbricht, M. A. Brockmann, A. Aigner et al., “Expression and function of the receptor protein tyrosine phosphatase ζ and its ligand pleiotrophin in human astrocytomas,” *Journal of Neuropathology & Experimental Neurology*, vol. 62, no. 12, pp. 1265–1275, 2003.

[9] Y. Ma, F. Ye, X. Xie, C. Zhou, and W. Lu, “Significance of PTPRZ1 and CN185 expression in cervical carcinoma,” *Archives of Gynecology and Obstetrics*, vol. 284, no. 3, pp. 699–704, 2011.

[10] H. Makinoshima, G. Ishii, M. Koijima et al., “PTPRZ1 regulates calmodulin phosphorylation and tumor progression in small-cell lung carcinoma,” *BMC Cancer*, vol. 12, no. 1, p. 337, 2012.

[11] Z. Xia, D. Ouyang, Q. Li et al., “The expression, functions, interactions and prognostic values of PTPRZ1: a review and bioinformatic analysis,” *Journal of Cancer*, vol. 10, no. 7, pp. 1663–1674, 2019.

[12] R. Zhu, J. Xin-Chun, L. De-Yu, Z. Chao, and W. Yuan, “[Expression of PTPRZ1 in oral squamous cell carcinoma originated from oral submucous fibrosis and its clinical significance],” *Shang Hai Kou Qiang Yi Xue*, vol. 26, no. 2, pp. 198–203, 2017.

[13] D. Shang, X. Xu, D. Wang, Y. Li, and Y. Liu, “Protein tyrosine phosphatase ζ enhances proliferation by increasing β-catenin nuclear expression in VHL-inactive human renal cell carcinoma cells,” *World Journal of Urology*, vol. 31, no. 6, pp. 1547–1554, 2013.

[14] Y. Jiang, L Liao, C Shrestha et al., “Reduced expression of E-cadherin and p120-catenin and elevated expression of PLC-y1 and PIKE are associated with aggressiveness of oral squamous cell carcinoma,” *International Journal of Clinical and Experimental Pathology*, vol. 8, no. 8, pp. 9042–9051, 2015.

[15] R. C. Schackmann, M Tenhagen, R. A van de Ven, and P. W Derksen, “p120-catenin in cancer - mechanisms, models and opportunities for intervention,” *Journal of Cell Science*, vol. 126, no. Pt 16, pp. 3515–3525, 2013.

[16] L.-W. Ma, Z.-T. Zhou, Q.-B. He, and W.-W. Jiang, “Phosphorylated p120-catenin expression has predictive value for oral cancer progression,” *Journal of Clinical Pathology*, vol. 65, no. 4, pp. 315–319, 2012.

[17] S. Alemá and A. M. Salvatore, “p120 catenin and phosphorylation: mechanisms and traits of an unresolved issue,” *Biochimica et Biophysica Acta*, vol. 1773, no. 1, pp. 47–58, 2007.

[18] A. H. Huber, W. J. Nelson, and W. I. Weis, “Three-dimensional structure of the armadillo repeat region of β-catenin,” *Cell*, vol. 90, no. 5, pp. 871–882, 1997.

[19] K. W. Zaid, “Immunohistochemical assessment of E-cadherin and β-catenin in the histological differentiations of oral squamous cell carcinoma,” *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 20, pp. 8847–8853, 2014.

[20] B. J. MacDonald, K. Tamai, and X. He, “Wnt/β-Catenin signaling: components, mechanisms, and diseases,” *Developmental Cell*, vol. 17, no. 1, pp. 9–26, 2009.

[21] M. Q. Soares, J. A. Mendonça, M. O. Moral, C. R. Leles, A. C. Batista, and E. F. Mendonça, “E-cadherin, β-catenin, and αββ and α3β1 integrin expression in primary oral squamous cell carcinoma and its regional metastasis,” *Histology & Histochemistry*, vol. 30, no. 10, pp. 1213–1222, 2015.

[22] J. Chuerdangphu, T. Ekalaksananan, P. Chaiyarit et al., “Effects of arcACHINE on proliferation of oral squamous cell carcinoma cells by dysregulating c-Myc and miR-22, directly targeting oncostatin M,” *PLoS One*, vol. 13, no. 1, Article ID e0192009, 2018.

[23] C. F. Wang, H. C Zhang, X. M Feng, X. M Song, and Y. N Wu, “Knockdown of MS11 inhibits the proliferation of human oral squamous cell carcinoma by inactivating STAT3 signaling,” *International Journal of Molecular Medicine*, vol. 44, no. 1, pp. 115–124, 2019.

[24] L. Zhang, M. Gallup, L. Zlock, W. E. Finkbeiner, and A. N. McNamara, “Rac1 and Cdc42 differentially modulate cigarette smoke-induced airway cell migration through p120-catenin-dependent and -independent pathways,” *American Journal of Pathology*, vol. 182, no. 6, pp. 1986–1995, 2013.

[25] V. Karkhanis, L. Alinari, H. G. Ozer et al., “Protein arginine methyltransferase 5 represses tumor suppressor miRNAs that down-regulate CYCLIN D1 and c-MYC expression in aggressive B-cell lymphoma,” *Journal of Biological Chemistry*, vol. 295, no. 5, pp. 1165–1180, 2020.

[26] K. Meng, A. Rodriguez-Pena, T. Dimitrov et al., “Pleiotrophin signals increased tyrosine phosphorylation of beta –catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta,” *Proceedings of the National Academy of Sciences*, vol. 97, no. 6, pp. 2603–2608, 2000.

[27] E. Stepniak, G. L. Radice, and V. Vasioukhin, “Adhesive and signaling functions of cadherins and catenins in vertebrate development,” *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 5, Article ID a002949, 2009.

[28] Y. Zhang, H. Jiao, Y. Wu, and X. Sun, “P120-catenin regulates pulmonary fibrosis and TGF-β induced lung fibroblast differentiation,” *Life Sciences*, vol. 230, pp. 35–44, 2019.

[29] J. P. MacKeigan, L. O. Murphy, and J. Blenis, “Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance,” *Nature Cell Biology*, vol. 7, no. 6, pp. 591–600, 2005.

[30] K. Di-Szachnowicz, “Protein tyrosine phosphatase receptor R and Z1 expression as independent prognostic indicators in oral squamous cell carcinoma,” *Head & Neck*, vol. 37, no. 12, pp. 1816–1822, 2015.

[31] L. Li, S. Ji, C. Shrestha et al., “p120-catenin suppresses proliferation and tumor growth of oral squamous cell carcinoma via inhibiting nuclear phospholipase C-γ1 signaling,” *Journal of Cellular Physiology*, vol. 235, no. 12, pp. 9399–9413, 2020.

[32] T. -L. Yan, M. Wang, Z. Xu et al., “Up-regulation of syncytin-1 contributes to TNF-α-enhanced fusion between OSCC and HUV-ECs partly via Wnt/β-catenin-dependent pathway,” *Scientific Reports*, vol. 7, no. 1, p. 40983, 2017.

[33] Y. Feng, F. R. Dehmerhi, W. Xiao et al., “Interdependency of EGF and GLP-2 signaling in attenuating mucosal atrophy in a mouse model of parenteral nutrition,” *Cellular and Molecular Biology Journal of Oncology*.
Gastroenterology and Hepatology, vol. 3, no. 3, pp. 447–468, 2017.

[34] Y.-T. Liu, D. Shang, S. Akatsuka et al., “Chronic oxidative stress causes amplification and overexpression of ptprz1 protein tyrosine phosphatase to activate β-catenin pathway,” American Journal Of Pathology, vol. 171, no. 6, pp. 1978–1988, 2007.

[35] C. R. McClain, F. J. Sim, and S. A. Goldman, “Pleiotrophin suppression of receptor protein tyrosine phosphatase-β maintains the self-renewal competence of fetal human oligodendrocyte progenitor cells,” Journal of Neuroscience, vol. 32, no. 43, pp. 15066–15075, 2012.

[36] P. G. Gritsenko, N. Atlasy, C. E. J. Dieteren et al., “p120-catenin-dependent collective brain infiltration by glioma cell networks,” Nature Cell Biology, vol. 22, no. 1, pp. 97–107, 2020.

[37] N. Ishiyama, S.-H. Lee, S. Liu et al., “Dynamic and static interactions between p120 catenin and E-cadherin regulate the stability of cell-cell adhesion,” Cell, vol. 141, no. 1, pp. 117–128, 2010.

[38] R. Hernández-Martínez, N. Ramkumar, and K. V. Anderson, “p120-catenin regulates WNT signaling and EMT in the mouse embryo,” Proceedings of the National Academy of Sciences of the United States of America, vol. 116, no. 34, pp. 16872–16881, 2019.

[39] U. Ulbricht, C. Eckerich, R. Fillbrandt, M. Westphal, and K. Lamszus, “RNA interference targeting protein tyrosine phosphatase ζ/receptor-type protein tyrosine phosphatase β suppresses glioblastoma growth in vitro and in vivo,” Journal of Neurochemistry, vol. 98, no. 5, pp. 1497–1506, 2006.

[40] X. Ding, X. Wang, S. Lu, X. Gao, and S. Ju, “P120-Catenin and its phosphorylation on Tyr228 inhibits proliferation and invasion in colon adenocarcinoma cells,” OncoTargets and Therapy, vol. 12, pp. 10213–10225, 2019.

[41] Y. Liu, Q.-Z. Dong, Y. Zhao et al., “P120-catenin isoforms 1A and 3A differently affect invasion and proliferation of lung cancer cells,” Experimental Cell Research, vol. 315, no. 5, pp. 890–898, 2009.

[42] Y. Liu, Q.-C. Li, Y. Miao et al., “Ablation of p120-catenin enhances invasion and metastasis of human lung cancer cells,” Cancer Science, vol. 100, no. 3, pp. 441–448, 2009.