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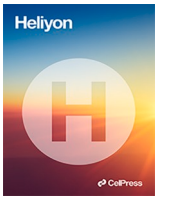

Review article

Mechanisms and markers of malignant transformation of oral submucous fibrosis

Fen Lin [a], Ting Xiao [b], Baisheng Wang [b], Liping Wang [b], Gui Liu [b], Rifu Wang [b], Changqing Xie [b,c,*], Zhangui Tang [b,**]

[a] *Hospital of Stomatology, Zhongshan city, Zhongshan, Guangdong 528400, China*
[b] *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 410008, Hunan, China*
[c] *Postdoctoral Research Workstation, Cancer Research Institute and School of Basic Medicine, Central South University, Changsha 410078, Hunan, China*

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ABSTRACT

Oral submucous fibrosis (OSF) is a chronic premalignant disease associated with betel quid chewing. Epidemiological studies indicate that there are approximately 5 million individuals suffering from OSF worldwide, with a concerning malignancy transformation rate of up to 4.2 %. When OSF progresses to oral squamous cell carcinoma (OSCC), the 5-year survival rate for OSCC drops to below 60 %. Therefore, early screening and diagnosis are essential for both preventing and effectively treating OSF and its potential malignant transformation. Numerous studies have shown that the malignant transformation of OSF is associated with various factors, including epigenetic reprogramming, epithelial-mesenchymal transition, hypoxia, cell cycle changes, immune regulation disturbances, and oxidative damage. This review article focuses on the unraveling the potential mechanisms underlying the malignant transformation of OSF, as well as the abnormal expression of biomarkers throughout this transformative process, with the aim of aiding early screening for carcinogenic changes in OSF. Furthermore, we discuss the significance of utilizing blood and saliva components from patients with OSF, along with optical diagnostic techniques, in the early screening of OSF malignant transformation.

1. Introduction

Oral submucosal fibrosis (OSF) is a chronic and progressive precancerous condition affecting the oral mucosa [1]. It is caused by several factors, including betel nut and tobacco chewing [2–4], potential immunological factors [5,6], vitamin B and C deficiencies [7,8], iron and copper micronutrient deficiencies [7,9,10], gene mutation [11], and spicy food consumption [12] (Table 1) [13–17]. According to epidemiological surveys, OSF is prevalent in south and Southeast Asian countries [18–22], with high-incidence areas like

* Corresponding author. 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 410008, Hunan, China.
** Corresponding author.
E-mail addresses: xiechangqing0226@126.com (C. Xie), zhgtang@csu.edu.cn (Z. Tang).

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China and India reporting incidence rates of 0.9–4.7 % [4] and 0.62–6.42 % [23] respectively. Patients with OSF typically exhibit limited or diffuse gray-white changes, oral mucosal roughness, and palpable fibrous bands (Fig. 1a) [1,24,25]. The pathological changes associated with OSF affect two regions: epithelial layer and connective tissue (Table 2) [26].

OSF belongs to a group of the oral potentially malignant disorders (OPMD) (Figs. 1b and 2(a - e)). Like other types of cancer, early screening and diagnosis can significantly improve the prognosis. Understanding the mechanism of malignant transformation in OSF and identifying abnormally expressed biomarkers during this process can help clinicians in achieving early diagnosis [27]. To this end, we review the current potential mechanisms underlying malignant transformation in OSF and the biomarkers typically associated with these transformative processes (Table 3).

Potential mechanisms and associated biomarkers in the malignant transformation of OSF.

As a precancerous condition, the progression of OSF to oral squamous cell carcinoma (OSCC) is influenced by various factors, including betel quid chewing, smoking, drinking, and microbial infection [60]. Notably, the risk of malignant transformation increases when OSF is accompanied by other OPMDs, such as leukoplakia, erythroplakia, and lichen planus. According to epidemiological surveys, the likelihood of malignant transformation in OSF is estimated at 4.2 % (95 % CI 2.7–5.6 %) [61]. Mechanistically, current research indicates that the malignant transformation mechanism of OSF [62] is primarily associated with epigenetic changes [63], epithelial–mesenchymal transition (EMT) [64], cell cycle alterations [65], hypoxia [66], immunomodulatory disorders [67–70], and oxidative injury [71]. In this context, we have identified specific biomarkers that are abnormally expressed during the malignant transformation of OSF, offering insights into the mechanisms underlying this transformation (Fig. 3).

Traditional disease diagnosis primarily relies on clinical manifestations, imaging findings, histopathology, etc. However, in recent years, the advancements in molecular biology diagnostic technologies have led to surge in data, revealing variations in the levels of numerous molecular biomarkers in patients. These variations can be used for early disease detection, auxiliary diagnosis, guided targeted therapies, and prognostic assessment [27]. By assessing the differences in the expression of biomarkers in vivo, it is possible to predict and monitor the potential occurrence of malignant transformation in these precancerous lesions. Owing to the particularity of oral anatomy, in addition to the conventional method of pathological biopsy of affected tissue, emerging tools for identifying malignant transformation in OSF include the examination of saliva, crevicular fluid, and plaque, as well as the application of optical diagnostic techniques.

1.1. Epigenetic changes

The occurrence of oral cancer is thought to be associated with epigenetic changes, with a primary focus on the inactivation of tumor suppressor genes and the activation of protooncogenes [63]. One such tumor suppressor gene, phosphatase and tensin homolog (*PTEN*), plays a crucial role in inhibiting uncontrolled cell growth and cell division, regulating signaling pathways, and inducing apoptosis [28]. Similarly, *P16, P53*, and *P63* are all tumor suppressor genes [29–31]. When a tumor suppressor gene is mutated, it can continuously promote cell proliferation and contribute to tumorigenesis. The protooncogene *c-Myc* (gene locus: 8q24.12-q24.13) is activated in tumors and promotes tumor development by regulating cell proliferation. It can inhibit the activation of *P53* by inducing the expression of auxin response factor tumor suppressor. *P53*, in turn, represses *c-Myc* through microRNA-mediated mechanisms [72]. Notably, *c-Myc* is significantly overexpressed in cases of OSCC accompanied by OSF [32].

Epigenetic regulatory processes, including promoter cytosine-phosphate-guanine methylation and histone modifications, have been identified as key events in cancer development [73]. Aberrant promoter methylation leads to the transcriptional silencing of tumor suppressor genes, contributing to cellular malignant transformation and tumorigenesis [74]. The Wnt/β-catenin pathway is one of the most critical pathways implicated in the development of various cancers, including OSCC and OSF. The downregulation and methylation of Wnt inhibitor-1 (WIF1), a Wnt antagonist, have been observed in many malignancies. A study revealed frequent WIF1 methylation in OSCC cases with areca-chewing habits, while this methylation was not detected in normal oral mucosa and OSF tissues at different stages, suggesting that WIF1 methylation is tumor-specific in the context of OSF [40]. Relatively few studies have explored histone modification in OSF carcinogenesis. However, in both OSF and OSCC, histone modification may play a role in gene regulation by affecting the affinity of other transcription factors with structural gene promoters [75,76]. For example, histone deacetylase 1 can inhibit the proliferation of OSCC cells by reducing the stability of proliferating cell nuclear antigen (PCNA) mRNA to regulate its expression [76]. Drugs that inhibit histone modifications are currently in clinical use for cancer treatment [77]. For example, 7-(4-(3-ethynylphenylamino)-7-methoxyquinazolin-6-yloxy)-N-hydroxyheptanamide is a histone deacetylase inhibitor under evaluation in clinical trials for the treatment of advanced solid tumors, including head and neck cancers [78].

In addition to genetic mutations, growing lines of evidence indicate the involvement of non-coding RNAs (ncRNAs), which do not

Table 1
Comprehensive list of etiological factors of OSF.

Etiology		References
Immunologic causes	Inflammation, autoimmunity	[5,6]
Chronic irritation	Chewing betel nuts, Chewing Tobacco, Chewing clay, Chewing fennel seeds, Drinking and smoking, Eating spicy food.	[2–4,12,14,15,17]
Trace Element Deficiency	Deficiency of vitamin B, C, Deficiency of micronutrient such as iron and copper	[7–10]
Genetic factor	gene mutation	[11]
Others	Alkaline injury	[16]

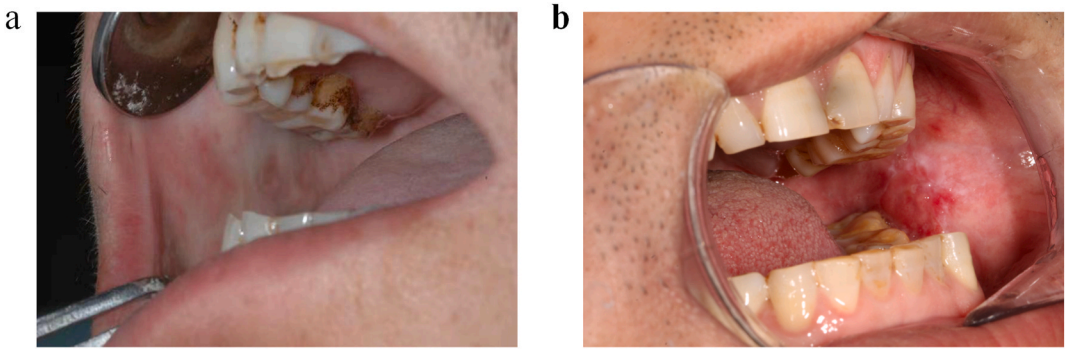

Fig. 1. (A) Oral submucosal fibrosis. Fibrosis of the left buccal area characterized by grayish-white mucosal alterations and palpable fibrous striated changes. (b) Oral squamous carcinoma with oral submucosal fibrosis. Fibrosis of the right buccal area displaying grayish-white mucosal changes and ulcerated surfaces in the posterior cheek. This conditions presents with a hard base and an indistinct boundary upon touch. This study received prior approval from the Ethics Committee of Xiangya Stomatological Hospital of Hunan Central South University, China. The study was conducted in compliance with established ethical guidelines and with the informed consent of participants. (Original figures).

Table 2
The four stages of pathologic changes in oral submucous fibrosis.

Stage	Pathologic manifestations
Earliest	Some fine collagen fibers appear, and there is evident edema, and the blood vessels are sometimes dilated and congested with neutrophil infiltration.
Early	There is a band of vitreous degeneration of collagen fibers immediately below the epithelium, and below that there is inter-collagen fiber edema with lymphocytic infiltration.
Middle	There are moderate vitreous changes of collagen fibers with mild edema and lymphocytic and plasma cell infiltration.
Late	All collagen fibers are glassy with narrowing or occlusion of blood vessels. Epithelial atrophy is present with shortened or absent epithelial spikes. Some epithelia appear hyperplastic, with hypertrophy of the epithelial spikes and vacuoles in the epithelium, while others show abnormal hyperplasia. Massive muscle fiber necrosis can be seen in the tissues of patients, with severely impaired mouth openings.

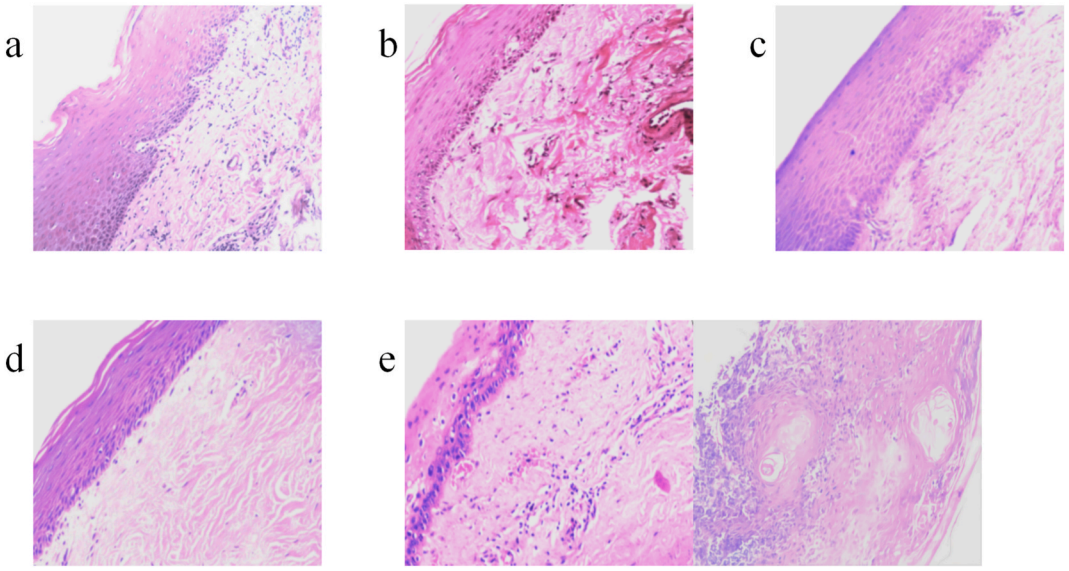

Fig. 2. Hematoxylin-eosin staining was performed on samples from patients with oral submucosal fibrosis and oral squamous cell carcinoma with oral submucosal fibrosis. (a) The lesion at the earliest stage. Some fine collagen fibers appear, with obvious edema accompanied by neutrophil infiltration (hematoxylin–eosin, × 100). (b) The lesion in its early stage. A band of vitreous degeneration can be seen in collagen fibers immediately below the epithelium, along with inter-collagen fiber edema with lymphocytic infiltration (hematoxylin–eosin, × 100). (c) The lesion in the middle stage. Moderate vitreous changes can be observed in collagen fibers, along with mild edema and lymphocytic and plasma cell infiltration (hematoxylin–eosin, × 100). (d) The lesion in its late stage. All collagen fibers exhibit a glassy appearance with noticeable narrowing or occlusion of blood vessels (hematoxylin–eosin, × 100). (e) Oral squamous cell carcinoma with oral submucosal fibrosis (hematoxylin–eosin, × 100). This study received a prior approval from the Ethics Committee of Xiangya Stomatological Hospital of Hunan Central South University, China. The study was conducted in compliance with established ethical guidelines and with the informed consent of participants. (Original figures).

Table 3
Biomarkers associated with malignant transformation of OSF.

Biomarker	Expression	Potential mechanisms	Sample Size	Methods	References
PTEN	↑	Epigenetic Changes	30 OSF, 30 OSCC, 10 normal	Immunohistochemistry	[28]
P63	↑	Epigenetic Changes	32 OSCC, 13 control, 19 oral pre-cancer with dysplastic, 17 oral pre-cancer with non-dysplastic	Immunohistochemistry, Western blot, RT-PCR	[29]
P53	↑	Epigenetic Changes	21 OSF, 21 OSCC , 6 OSCC-OSF	Immunocytochemistry, PCR-single-strand conformation polymorphism	[30]
P16	↑	Epigenetic Changes	10 normal, 30 OSF, 30 OSCC	Immunohistochemistry	[31]
c-Myc	↑	Epigenetic Changes	68 OSF, 10 normal	Immunohistochemistry	[32]
miR-21	↑	Epigenetic Changes	20 OSF, 20 OSCC, 40 normal	RT-PCR	[33]
LncRNA ADAMTS9-AS2	↓	Epigenetic Changes	10 normal, 10 OSF, 20 OSCC	RT-PCR	[34]
β1 integrins	↑	EMT	15 normal, 81 OSF, 16 OSCC-OSF	Immunohistochemistry	[35]
MMP-9	↑	EMT	192 OSCC, 73 OSF, 191 healthy areca chewers	PCR-based restriction fragment length polymorphism analysis	[36]
MMP-12	↑	EMT	30 OSCC, 30 OSF, 30 normal	Enzyme-linked immunosorbent assay	[37]
DKK3	↑	EMT	55 OSCC, 45 OSF, 15 normal	Immunohistochemistry, RT-PCR	[38]
SFRPs	↓	EMT	55 OSCC, 45 OSF, 15 normal	Immunohistochemistry, RT-PCR, Methylation-specific PCR	[39]
WIF1	↓	EMT	55 OSCC, 45 OSF, 15 normal	Immunohistochemistry, RT-PCR	[40]
α-SMA	↑	EMT	30 normal, 50 OSF, 105 OSCC-OSF	Immunohistochemistry	[41]
osteopontin	↑	EMT	20 normal, 40 OSF, 40 OSCC	Immunohistochemistry	[42]
PCNA	↑	Cell Cycle Alterations (proliferation)	30 OSF, 10 OSCC	Immunohistochemistry	[43]
hTERT	↑	Cell Cycle Alterations (proliferation)	5 OSCC-OSF, 10 normal, 20 OSF, 15 OSCC,	Immunohistochemistry	[44]
Ki67	↑	Cell Cycle Alterations (proliferation)	30 normal, 50 OSF, 105 OSCC-OSF.	Immunohistochemistry	[41]
cyclin D1	↑	Cell Cycle Alterations (proliferation)	51 OSCC, 4 OSF, 12 normal	Immunohistochemistry, Slot-blot	[45]
survivin	↑	Cell Cycle Alterations (apoptosis)	10 normal, 40 OSF, 42 OSCC-OSF	Immunohistochemistry, Western blot, Immunoprecipitation	[46]
caspase-3	↓	Cell Cycle Alterations (apoptosis)	15 normal, 81 OSF, 16 OSCC-OSF	Immunohistochemistry	[47]
HIF-1	↑	Hypoxia and Neoangiogenesis	100 OSCC, 100 control, 100 OSF	RT-PCR, Enzyme linked immunosorbent assay	[48]
VEGF	↑	Hypoxia and Neoangiogenesis	100 OSF, 100 OSCC, 100 control	RT-PCR, Enzyme linked immunosorbent assay	[48]
CD105	↑	Hypoxia and Neoangiogenesis	30 normal, 50 OSF, 105 OSCC-OSF.	Immunohistochemistry	[41]
ET-1	↑	Hypoxia and Neoangiogenesis	15 OSF, 15 OSCC, 15 normal	Enzyme linked immunosorbent assay	[49]
PAI-1	↑	Hypoxia and Neoangiogenesis	17 normal, 6 oral epithelial dysplasia, 43 OSCC; 6 normal, 25 OSF;	Immunohistochemistry, Western blot	[50,51]
PD-1/PD-L1	↑	Immunomodulatory Disorder	44 OSCC-OSF, 44 OSCC, 3 normal	Immunohistochemistry, Double immunofluorescence labeling, RT-PCR	[52]
CD1a+	↓	Immunomodulatory Disorder	14 OSF, 45 OSCC, 8 normal, 9 OSCC-OSF	Immunohistochemistry	[53]
CICs	↑	Immunomodulatory Disorder	30 OSCC, 30 normal, 30 OPMD (OSF)	Spectrophotometric	[54]
Th17/Treg	↑	Immunomodulatory Disorder	30 normal, 72 OSF, 90 OSCC	Immunohistochemistry, Flow cytometry	[55]
8-OHdG	↑	Oxidative Injury	40 OSCC, 40 OSF, 40 control	Enzyme linked immunosorbent assay, High performance liquid chromatography	[56]
NO	↑	Oxidative Injury	29 OSF, 29 OSCC, 9 control	Modified copper-cadmium reduction	[57]
ceruloplasmin	↑	Oxidative Injury	50 OSF, 50 OSCC, 50 healthy areca chewers or tobacco, 50 healthy Control	Diagnostic kit- SensIT ceruloplasmin	[58]
SOD	↓	Oxidative Injury	29 OSF, 29 OSCC, 9 control	Modified copper-cadmium reduction	[57]
Vitamin C, Vitamin E	↓	Oxidative Injury	40 OSCC, 40 OSF, 40 control	Enzyme linked immunosorbent assay, High Performance Liquid Chromatography	[56]
SASP components (IL-1, IL-6 and GRO-α)	↑	Oxidative Injury (senescence)	10 OSF, 5 normal	Immunohistochemistry	[59]

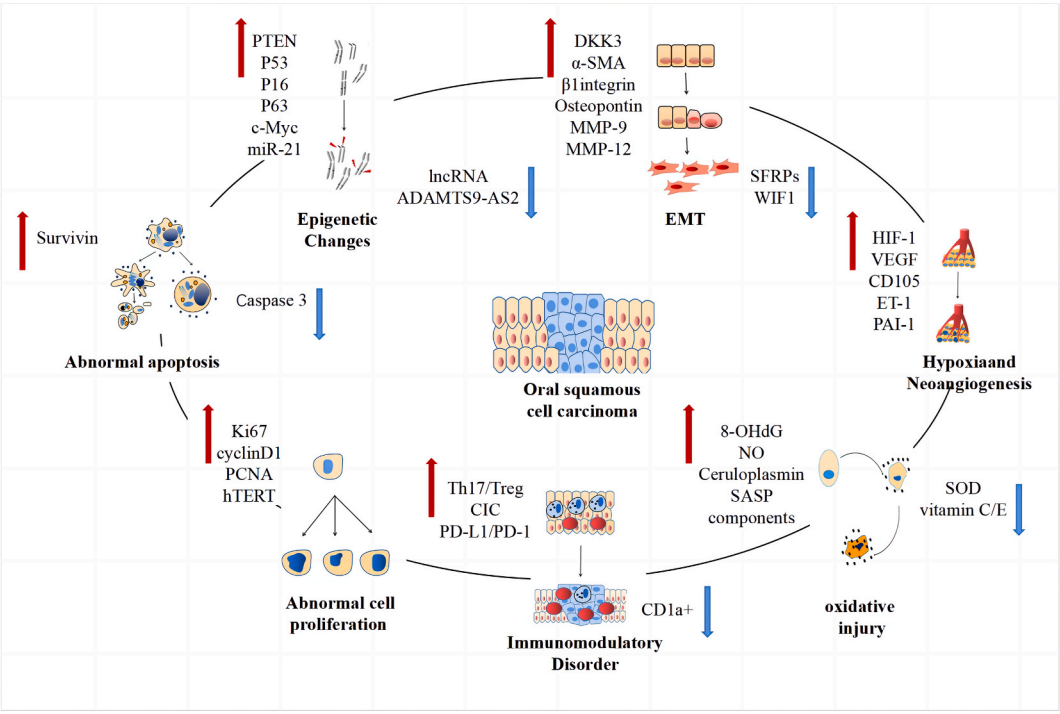

Fig. 3. The mechanisms and markers of the malignant transformation of oral submucosal fibrosis. The process of the malignant transformation of oral submucosal fibrosis into oral squamous cell carcinoma has been associated with epigenetic modifications, epithelial–mesenchymal transition, cell cycle alterations, hypoxia and neoangiogenesis, immunomodulatory disturbances, and oxidative damage. In the diagram, the black arrow represents inclusion or grading, the red arrow represents rising, and the blue arrow represents falling.

encode proteins, in the malignant transformation of OSF. They play a key role in physiological and pathological processes by regulating gene transcription and translation through various mechanisms, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) [79]. LncRNAs can interact with miRNAs as competitive endogenous RNAs, participating in the post-transcriptional regulation of target genes and modulating the expression of fibrosis and tumor-related genes [80]. For instance, lncRNA ADAMTS9-AS2 inhibits the growth, migration, and invasion of OSCC cells by interacting with miRNAs, thereby affecting significantly enriched pathways, including the metabolic, phosphatidylinositol 3-kinase/protein kinase B signaling pathway, and cancer pathways [34]. However, miRNA-21 can inhibit the expression of specific genes by binding to the *PTEN* gene, which, in turn, inhibits the Akt pathway and reduces the invasive and proliferative abilities of tumor cells [33,81].

1.2. Epithelial–mesenchymal transition (EMT)

EMT is a complex biological process that plays an indispensable role in tumorigenesis and metastasis. EMT typically occurs when epithelial cells lose their polarity, have their cell-to-cell junctions disrupted, and acquire the ability to invade and migrate [64].

During the process of chewing betel quid, the active ingredients can cause physical and chemical damage to epithelial cells. In response, stem cells located in the basal layer proliferate and differentiate to repair the damage, with a significant overexpression of β1 integrins. β1 integrin is a transmembrane protein that connects the cytoskeleton to the extracellular matrix and is typically overexpressed in OSF and OSCC. Immunohistochemical staining shows that the expression of β1 integrins and osteopontin is typically higher in OSCC with OSF tissues [35]. Furthermore, osteopontin can bind to the integrin family through the Arg-Gly-Asp sequence, forming focal adhesion complexes [42], which, in turn, initiate multiple signal transduction pathways that promote cell adhesion, proliferation, and migration.

Additionally, matrix metalloproteinases can disrupt the histological barrier by degrading various protein components in the extracellular matrix, thereby creating physical space for cell movement. During the malignant transformation of OSF, the overexpression of matrix metalloproteinase (MMP)-9 and MMP-12 is associated with the disruption of the basement membrane and a propensity for cells to infiltrate the underlying matrix [37,36]. The destruction of the basement membrane further exacerbates the EMT progression and enhances heterogeneous cell invasion [82].

EMT involves multiple signaling pathways during the malignant transformation of OSF. Our previous studies have shown that arecoline, an areca nut alkaloid, inhibits the ubiquitination degradation of proteasome activator (PA28 gamma) and activates downstream MEK/ERK signaling pathways in epithelial cells, enhancing the invasion and migration of epithelial cells by promoting EMT [83]. Dickkopf-3 (DKK3), an inhibitor of the Wnt signaling pathway, inhibits Wnt/β-catenin signaling by binding to Wnt ligands.

Notably, the expression of DKK3 increases significantly during the malignant transformation of OSF. An immunohistochemical analysis showed that DKK3 is primarily expressed in the epithelial cells of OSCC with OSF [38]. β-catenin can form a complex with E-cadherin, and this complex plays a crucial role in maintaining epithelial morphology and promoting cell adhesion [84,85]. Furthermore, β-catenin is a key protein of the Wnt/β-catenin signaling pathway. DKK3 inhibits the activity of β-catenin and prevented it from entering the nucleus, thereby inhibiting the Wnt/β-catenin signaling pathway. At the same time, DKK3 also reduces the stability of β-catenin/E-cadherin complex, leading to decreased cell adhesion and promoting tumor invasion and metastasis [86]. DDK3 may deregulate Wnt signaling, P53 signaling, apoptosis, Ca^{2+} signaling and mitochondrial signaling pathways in OSCC pathogenesis [83], suggesting its potential involvement in the malignant transformation of OSF.

Secreted frizzled-related proteins (SFRPs) and WIF1 can also disrupt the Wnt signaling pathway by binding to Wnt receptors, influencing fibrosis and its malignant transformation [40,39]. During the course of OSF carcinogenesis, SFRP1 and SFRP5 exhibit downregulated levels, with significant changes in their subcellular localization from the nucleus to the cytoplasm. Interestingly, WIF1 can interact with β-catenin, decreasing its translocation into the cell nucleus and thus preventing fibrotic events [87–89]. Mechanistically, the decreased expression of SFRP and WIF1 is attributed to a cytokine that promotes the methylation of SFRP and WIF1, thereby accelerating fibrosis and cancer progression [40,39].

Additionally, the Wnt/β-catenin signaling pathway can crosstalk with the TGF-β/Smad signaling pathway [90]. Arecoline can enhance the secretion of α-smooth muscle actin (α-SMA) by activating the TGF-β/Smad pathway [91]. The development and malignant transformation of OSF result from the interplay of various cells and molecular regulatory networks within the fibrotic microenvironment. Myofibroblasts express α-smooth muscle actin, which promotes cell contraction and accelerates the process of EMT [92]. The number of myofibroblasts continues to increase from the early to the later stages of OSF, suggesting that myofibroblasts could serve as an indicator to evaluate the severity of OSF [93]. In the early stages of epithelial tumor growth, cancer cells secrete growth factors, leading to the transformation of normal stroma into a "reactive stroma." These growth factors promote the differentiation of fibroblasts/myofibroblasts into cancer-associated fibroblasts [94], which may promote tumor progression by stimulating cancer cell growth, reducing cancer cell death, and activating proteolysis in the tumor stroma. This process can weaken the barrier for cancer cells, facilitating invasion and metastasis. Fibroblasts acquire a contractile phenotype, characterized by the formation of microfilament bundles and de novo expression of α-smooth muscle actin [95]. Studies have found high expression of α-SMA in OSF with abnormal epithelial proliferation [41,96], suggesting the likely involvement of α-SMA in the malignant transformation of OSF.

1.3. Cell cycle alterations

Cell proliferation and apoptosis are both important cellular activities for maintaining homeostasis [65]. In addition to physical friction and chemical stimulation, chewing betel nut places the oral mucosa in a state of constant pathological inflammatory stimulation [97,98]. At the same time, the reactive oxygen species (ROS) generated during oxidative stress can damage DNA and mitochondria, leading to abnormal programmed cell death [99–101]. For example, arecoline can induce the downregulation of genes, such as *P53*, *P21*, and *P27*, resulting in uncontrolled cell growth and inhibition of normal apoptotic processes, which, in turn, can lead to immortal cell proliferation and ultimately to the cancerous transformation of OSF [102,103].

By observing proteins associated with cell proliferation, such as proliferating cell nuclear antigen (PCNA), which is barely detectable in the G0-G1 phase of the cell cycle, expression increases significantly from the late G1 phase. Compared with normal tissues, PCNA is highly expressed in OSF and OSCC tissues [43]. Some other cell proliferation proteins, such as Ki67 [41,96,104] and cyclin D1 [45], are highly expressed in OSF with high-risk epithelial dysplasia. The abnormal proliferation within OSF can be monitored for potential cancerization by assessing the expression of cell proliferation-related proteins.

Telomerase consists of a human telomerase reverse transcriptase (hTERT) unit and an RNA unit [105]. Telomeres play an important role in maintaining chromosomal stability and cell viability. Telomerase can lengthen shortened telomeres, which have limited replication ability, thereby enhancing the proliferative capacity of cells in vitro. Telomerase activity is typically inhibited in normal human tissues but is reactivated in tumors, potentially contributing to the process of malignant transformation. OSF and OSCC cells have been found to exhibit significantly higher expression of hTERT compared to normal tissues [44].

Classical apoptosis is a precisely regulated process in the body. It begins with the sensing of apoptosis-related signal molecules by signal receptors. Subsequently, B cell lymphoma protein-2 (Bcl-2) proteins integrate the apoptotic signals, leading to the activation of caspases proteins that execute cellular apoptosis. An immunohistochemical analysis revealed that the expression of caspase-3 protein was hardly detectable in OSCC compared to OSF [47]. However, other studies have indicated that there is no difference in the expression of the Bcl-2 protein upstream of caspase-3 in OSF and OSCC [106]. We speculate that this result may be related to the limited sample size in this study. Additionally, the expression of the apoptotic protein inhibitor, survivin, is significantly increased in OSF and OSCC with OSF, compared to its almost undetectable levels in normal tissues [46]. When proliferating transitional cells are not promptly removed by the body, tumors can begin to develop.

1.4. Hypoxia and neoangiogenesis

During OSF development, the physical and chemical stimulation caused by chewing betel nut can lead to atrophy and reduction of blood vessels, resulting in local tissue hypoxia in OSF [66]. This hypoxia, in turn, stimulates the significant release of cytokines in the tissue microenvironment and stimulates the growth of new blood vessels [107].

Hypoxia-inducible factor-1 (HIF-1) is commonly found in mammals and humans under hypoxic conditions. It targets downstream genes, such as vascular endothelial growth factor (VEGF), through transcription regulation, thereby promoting the formation of new

blood vessels [48]. Endoglin (CD105) is also upregulated in response to hypoxia and plays a crucial role in the formation of new blood vessels during tumor development [41,96]. As the disease progresses, the local microenvironment vasculature changes from being dilated in the earliest stages to becoming reduced and occluded in the later stages when cells experience hypoxic conditions, accompanied by an increase in and endothelin-1 (ET-1) secretion. For example, ET-1 can bind to its receptor and increase the production of hypoxia-inducible factor-1α (HIF-1α), which, in turn, induces the expression of VEGF and promotes angiogenesis [49]. In a state of continuous hypoxia, the local tissue of OSF is exposed to an environment with a constant influx of new blood vessels. When the number of new blood vessels is not regulated, it can lead to local malignant transformation [66].

However, hypoxia induces to cells secrete more cytokines, such as plasminogen activator inhibitor-1 (PAI-1) [50,51,108,109], which upon activation reduces blood plasmin function and inhibits fibrinolysis, leading to increased ECM production and accumulation. Moreover, excessive PAI-1 can aggravate fibrosis. In this scenario, the body continuously secretes cytokines, which enhance ECM accumulation and fibrosis, thus creating a vicious cycle.

To address the issue of local tissue hypoxia during OSF development, researchers have discovered that certain natural antifibrotic agents, such as capsaicin in spicy foods, can enhance endothelium-dependent vasodilation by upregulating endothelial nitric oxide synthase. This, in turn, helps alleviate hypoxia and inhibits fibrosis [110,111].

1.5. Immunomodulatory disorder

Numerous studies have reached a consensus that immune disorders are implicated in the onset of fibrosis [67–70]. Oral cancer development is associated with an imbalance in immune regulation that allows tumor cells to evade immune surveillance, and dysregulation of immune regulation may lead to the malignant transformation of OSF.

Programmed death-1 (PD-1) binds to programmed death ligand-1 (PD-L1), activating the src homology-2 protein tyrosine phosphatase in proximity to T cell receptor and cluster of differentiation 28 (CD28) signaling, thereby inhibiting T cells proliferation and activation [112]. A previous study showed that the expression of the corresponding cytokines PD-1/PD-L1 in the OSCC of the OSF group was significantly higher than in the OSCC group. This increase in PD-1/PD-L1 inhibits the differentiation and proliferation of T cells and promotes the apoptosis of activated cytotoxic lymphocytes [52].

Some scholars have proposed that the process of OSF malignant transformation can be attributed to local tissue immunosuppression [113,114]. A significant decrease in $CD1a^{+}$, a marker that specifically identifies immature dendritic cells and Langerhans cells, has been observed in patients with OSF, OSCC with OSF, and OSCC [53]. The authors speculate that dendritic cells may enter a suppressed state during the malignant transformation of OSF, and the reduced dendritic cell population prevents the timely elimination of abnormally proliferating cells, thereby increasing the likelihood of local tissue malignant transformation. Dendritic cells present antigens that bind to specific antibodies, forming immune complexes, which result in circulating immune complexes (CICs) [54]. The accumulation of CICs in vivo also indicates a reduction in dendritic cells. A peripheral blood sample analysis revealed significantly higher T helper type 17 (Th17)/regulatory T (Treg) expression in OSCC with OSF than in OSCC without OSF, with the Th17/Treg ratio gradually shifting towards Treg cells [55]. This increase in the number of Treg cells can suppress the immune response and promote the occurrence and development of tumors.

The malignant transformation of OSF is accompanied by the suppression of the immune system, which prevents the timely elimination of abnormally proliferating cells, thereby allowing tumor cells to escape immune surveillance.

1.6. Oxidative injury

As mentioned above, the cells in the OSF lesion area are exposed to a hypoxic microenvironment [71], which induces cell mitochondria to produce a significant amount of ROS. If not removed in time, it can lead to oxidative stress, resulting in the high expression of 8-hydroxydeoxyguanosine (8-OHdG) and nitric oxide (NO) in OSF. 8-OHdG is a potent oxidant generated in vivo that targets guanine bases within DNA molecules [115]. An analysis of saliva and blood composition in OSF and OSCC patients revealed elevated levels of 8-OHdG and NO [56,57]. Under normal conditions, the body maintains its internal environment and combats damage from ROS by producing antioxidants and eliminating damaged cells. Specifically, ceruloplasmin, an endogenous antioxidant, inhibits ROS production in the body. Compared to healthy individuals, patients with OSF and OSCC exhibit overexpression of serum ceruloplasmin [58]. While antioxidants, such as dismutase (SOD) and vitamin C, can scavenge ROS produced by the body, but patients with OSF and OSCC have been found to have significantly lower levels of SOD [57] and vitamin C/E [56] compared to their healthy counterparts [116]. Oxidative stress can damage the DNA of normal cells. Owing to mutations in genetic information or out-of-control epigenetic regulation, certain cells may acquire higher proliferative and migratory capabilities, ultimately leading to tumorigenesis.

Additionally, oxidative stress injury can induce fibroblasts to transform into a senescent myofibroblast phenotype, further accelerating ROS production, thus creating a vicious cycle [117]. On the one hand, senescent myofibroblasts can acquire an anti-apoptotic phenotype [118]. On the other hand, the secretion of senescence-associated secretory phenotype (SASP) components, such as IL-1, IL-6 and GRO-α, by myofibroblasts can lead to epithelial cell DNA damage, drive genetic instability [59,119–121], and promote the malignant transformation of OSF [119,122,123].

1.7. Future Prospects

In recent years, there has been a growing demand for the development of non-invasive techniques to detect malignant transformation of diseases. In the case of oral precancerous lesions, optical diagnostic techniques have gained prominence for detecting

structural and morphological abnormalities in local mucosa, enabling early detection of malignant transformation. However, when compared to the conventional method of pathological biopsy, the accuracy of these techniques for detecting early mucosal malignancy remains controversial. Moreover, exosomes and microvesicles, which are nano-sized membranous vesicle containing proteins, lipids, RNA, and other bioactive molecules, play a role in intercellular information transfer and hold potential for early detection of localized cancer and disease prognosis [124]. For example, salivary exosomal microRNA-24-3p has been found to facilitate the proliferation of OSCC cells by targeting period 1, which can serve as a potential novel diagnostic biomarker for OSCC [125].

The human body is a huge repository of microorganisms. Under normal circumstances, the oral microbial ecosystem remains relatively stable [126]. Among the factors contributing to cancer, microorganisms, including bacteria (e.g., *Helicobacter pylori*) and viruses (e.g., human papilloma virus) [127], play a significant role. Studies have revealed that disruptions in microbiota balance are associated with various diseases, and this dysbiosis can exert carcinogenic effects through several pathways, including the immune, inflammatory, and oxidative damage pathways [97,128]. Owing to the anatomical site specificity of oral carcinogenesis, it is closely associated with the balance of intraoral bacteria. Through metabolic analyses, researchers have identified alterations in the microbial metabolites associated with OSF and OSCC accompanying OSF [129]. For instance, *Porphyromonas gingivalis*-induced autophagy suppresses cell proliferation through G1 arrest in oral cancer cells [130]. Recent research has highlighted that microbiota imbalance allows certain pathogenic microorganisms to secrete bacterial toxins [131,132], which can damage cellular DNA, causing mutations resulting in abnormal cell proliferation and apoptosis. Simultaneously, local tissues consistently exposed to chronic inflammatory stimulation induced by bacterial toxins. Microbes can directly or indirectly contribute to malignant transformation of cells by manipulating signaling pathways, chronic inflammation, etc. It has been proposed that investigating the relationship between oral microbiota alterations and immune regulation disorders could serve as the starting point for understanding the mechanisms underlying the malignant transformation of OSF.

2. Conclusion

There is limited body of research on the mechanism of malignant transformation in OSF. We posit that this paper serves as a valuable entry point for investigating the process of malignant transformation in OSF through the examination of variations in biomarker levels among patients with OSF, OSCC, and OSCC with OSF. Furthermore, gaining a deeper insight into the relevant biomarkers and molecular mechanisms underlying the malignant transformation of OSF is crucial for early clinical intervention and effectively reducing the morbidity and mortality of OSF-derived OSCC. Of course, the malignant transformation of OSF is the result of multiple factors, and not all cases of OSF will become malignant. It is possible to prevent the further progression and malignancy of OSF lesions by adopting healthier habits and reducing exposure to carcinogenic factors, such as discontinuing the consumption of betel nuts. Additionally, when OSF is combined with other OPMDs, proactive treatment of these associated conditions may also reduce the risk of progression and malignant transformation of OSF [133–136].

Ethics statement

This study was reviewed and approved by the Ethic Committee of the Hunan Xiangya Stomatological Hospital of Central South University (Changsha, China), with the approval number: 20,210,018. All patients provided informed consent to participate in the study.

Data availability statement

Data included in article supplementary material/referenced in article.

CRediT authorship contribution statement

Fen Lin: Writing - review & editing, Writing - original draft, Conceptualization. **Ting Xiao:** Writing - review & editing, Conceptualization. **Baisheng Wang:** Writing - review & editing, Data curation. **Liping Wang:** Writing - review & editing, Formal analysis. **Gui Liu:** Writing - review & editing, Data curation. **Rifu Wang:** Writing - review & editing, Methodology. **Changqing Xie:** Writing - review & editing, Writing - original draft. **Zhangui Tang:** Writing - review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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