Genetic Aspects of Oral Submucous Fibrosis

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Contents

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9.1 Introduction

Oral cancer is a multistep process which may be preceded by oral potentially malignant disorders (OPMDs), such as oral submucous fibrosis (OSF) [1]. The high mortality and morbidity rates associated with oral cancer are because of late diagnosis [2]. Identification of newer techniques to diagnose the disease at the earliest stage is facilitated by advances in research, which include molecular and biochemical markers for early detection. Some of these markers reflect genetic and epigenetic changes [3]. Gene ontology and bioinformatics have been used to identify genes that are mutated, upregulated, or downregulated. However, there are no in silico studies in OSF.

Learning Goals

- To understand the genetic alterations in OSF and to examine the biomarkers that may help to identify populations at risk particularly among betel quid/areca nut chewers.

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9.2 Genetic Susceptibility and Gene Expression in Tissue/Organ Fibrosis

In systemic fibrotic conditions involving the liver, kidney, and lung, the association of genetic susceptibility is observed [4–7]. In cirrhosis of the liver, in addition to exogenous factors such as alcohol abuse and viral hepatitis, genetic predisposition significantly contributes to both cirrhosis and liver carcinoma [4]. Besides environmental factors, genetic factors involved in aging/senescence, cell-cell adhesion, and host defense contribute to the increased risk of pulmonary fibrosis [6]. Genetic susceptibility is also important in systemic sclerosis of the skin and internal organs [7].

9.3 Genetic Susceptibility in Oral Submucous Fibrosis (OSF)

Genetic susceptibility is important in the development of betel quid (BQ)-induced OSF. The mechanism involved in genetic susceptibility to OSF involves the combined effect of various genes.

9.3.1 Collagen 1A1 and Collagen 1A2 (COL1A1 and COL1A2) Gene

The connective tissue extracellular matrix (ECM) forms a three-dimensional scaffold for the cells and is involved in tissue homeostasis by components of ECM such as fibronectin, elastin, collagen, and nonfibrillar protein such as hyaluronan, proteoglycan, and glycoproteins [8]. Cancer progression is intensified by hypoxia and collagen-rich conditions [9]. Impairment of ECM function and ECM-cell interaction play an important role in diseases such as fibrosis, cancer, and osteoarthritis [8].

OSF is a collagen-related disorder having dense collagen deposition in the oral submucosa. Buccal mucosa when exposed to areca alkaloid, due to chewing BQ, accumulate collagen [10].

In OSF, there are alterations in collagen fiber bundle diameter, thickness, and distribution [11]. There is altered expression of type I and type III collagen fibers. In early OSF, and normal mucosa COLI is 85% and COL III 15%. However, as the disease progresses, type III collagen is gradually replaced by type I collagen, thereby leading to collagen I-predominant microenvironment [12].

The stiffness of oral mucosa in OSF is due to loss of procollagen type III, a predominance of collagen type I, and complete loss of collagen type IV [13]. Relative to normal buccal fibroblasts, more type I collagen mRNA and type I collagen trimers are seen in OSF fibroblasts [14].

The genes involved in collagen synthesis (transcription, translation, and posttranslational processing), degradation, and collagen cross-linking include collagen 1A1 and 1A2 (COL1A1 and COL1A2), collagenase-1 (COLase), transforming growth factor-beta (TGF-β), lysyl oxidase, and cystatin C (CST3). These genes are implicated in the pathogenesis of OSF. The constituents of areca nut, mainly arecoline and arecaidine, are found to interact with COL1A1, COL1A2, COLase, and lysyl oxidase (LOX) expressed in fibroblasts. Gene- and gene-environmental interactions explain the differences between individuals having low and high exposure to areca nut (AN). During the development of OSF, the microenvironment around oral fibroblasts can vary between low- and high-exposure groups. This discrepancy may result from fibroblast selection by BQ ingredients or inflammatory factors during the progression of OSF. A high proportion of the OSF risk can be attributed to the genetic component [15].

9.3.2 Matrix Metalloproteinases (MMPs)

MMPs constitute a family of neutral proteases which can degrade the ECM [16]. Twenty-eight human MMPs have been identified so far. These enzymes are classified as per their substrate specificity and structural similarities. Among them, a few important examples are collagenases (MMP-1) gelatinases (MMP-2 and MMP-9), stromelysin (MMP-3), and membrane-bound MMPs. MMPs regulate ECM proteolysis and process

several biologically active proteins such as cytokines, cell-surface proteins, chemokines, TGF-β 1, and other inflammation-related molecules that contribute to tissue fibrosis [16]. Many MMPs are expressed and activated in OSF patients as well as in head and neck squamous cell carcinoma (HNSCC) [17]. Gene polymorphisms in MMPs are suspected to influence gene expression in OSF.

9.3.3 Collagenase-1 (COLase-1, MMP-1)

Interstitial collagenase, also known as collagenase I, or MMP-1 belongs to a subgroup of the MMP family. It is the principal collagenase that cleaves collagen type I, II, III, VII, and X collagen. Collagenase I is important in photocarcinogenesis and photoaging [18]. Collagenase I is produced by various cells such as macrophages, stromal fibroblasts, endothelial cells, epithelial cells, and tumor cells [19]. It is secreted as a pro-collagenase and can be activated by many signaling pathways [20]. In OSF, MMP-1 activity is found to be lower compared to normal oral mucosa indicating a difference in collagen metabolism in patients. However, there is no statistically significant difference among different histological grades of OSF [21, 22]. Elevated expression of MMP-1 in stromal cells of OSF has also been reported [23]. Elevated expressions of MMP-1 have been reported in OSCC patients having BQ-chewing habit [24]. MMP-1 promoter region enhances its transcriptional activity and contributes to carcinogenesis and cancer metastasis. Choudhary et al. reported that single nucleotide polymorphisms (SNPs) in the MMP-1 promoter region are associated with the susceptibility of BQ chewers to HNSCC and OSF in India. Habitual BQ chewing and alcohol consumption enhance the expression of the 2G allele of MMP-1 genes in HNSCC and OSF patients [17]. Moreover, the 2G phenotype of the MMP-1 promoter is found in higher frequency in OSF and OSCC patients in comparison to controls [25].

9.3.4 MMP-2 (Gelatinase-A) and MMP-9 (Gelatinase-B)

MMP-2, also known as gelatinase-A, and MMP-9, also known as gelatinase-B, are Zn^{2+}-dependent endopeptidases having similar structures. MMP-2 is expressed by a wide variety of cell types in normal conditions, while MMP-9 is expressed in only a few cell types including trophoblasts, osteoclasts, leukocytes, dendritic cells, and precursors [26]. MMP-2 gene is located on chromosome 16q, while MMP-9 is on chromosome 20q. MMP-2 degrades proteins in ECM as well as the basement membrane. It degrades type I, type IV, type V, and type X collagen, elastin, laminin, fibronectin, elastin, and proteoglycans [27]. Mutations in MMP-2 disrupt its transcriptional activity resulting in its increased transcription. Individuals carrying CC genotype are found to express more MMP-2 proteins than individuals carrying TT or CT genotype [28]. Lin et al. assessed the MMP-2 genotype association with the risk of OSF and OSCC in 58 OSF cases, 121 OSCC cases, and 147 control cases. The subjects carrying CC genotype had twofold more risk in the development of OSF [29]. MMP-9 is known to degrade extracellular matrix components such as fibrillin, decorin, elastin type IV, V, XI, and XVI collagen, laminin, and gelatin. It also activates factors such as pro-TNF and pro-TGF [30]. In OSF, expressions of MMP-2, MMP-9, TIMP-1, and TIMP-2 are high compared to healthy oral mucosa [23]. MMP-9 expression has been analyzed in 432 patients and was found to be elevated in saliva, mucosa, and serum of patients in OPMDs compared to control [31]. Arecoline stimulated TIMP-1 expression, but reduced fibroblasts MMP-2 and MMP-9 in buccal mucosa [32].

PCR, RFLP of single nucleotide polymorphisms (SNP) genotyping reveals no significant difference in MMP-2 and MMP-9 polymorphism in OSF patients compared to healthy controls. T allele showed a significant association with increasing clinicopathological grades of HNSCC [17]. Tu et al. studied MMP-9 SNP in BQ-related OSCC, OSF, and non-diseased BQ chewers. Functional association of MMP-9-1562 C/T polymorphism with increased OSCC was seen in young BQ chewers. However, in the elder population, this association was not observed. No association was observed between the joint MMP-9 -1562 C>T and MMP-3 -1171 5A>6A functional polymorphisms and OSCC risk or patient survival [33].

9.3.5 MMP-3 (Stromelysin-1)

MMP-3 degrades basal membrane and collagen type II, V, IX, and X. It also induces activation of MMP-1 and MMP-9. MMP-3 gene is located on chromosome 11q22.3. Ye et al. reported 6A allele showing lower promoter activity than the 5A allele in vitro [34]. Tu et al. assessed the association of MMP-3 genotype with OSCC and OSF risk in 71 OSF patients, 150 OSCC patients, and 98 controls. They reported that the frequency of 5A genotype in the MMP-3 promoter was more in the OSF than in the control group; however, no significant differences were observed between the OSCC

and control groups. 5A genotype in MMP-3 promoter was found to be associated with OSF risk but not OSCC [35].

In the case-control study of 362 participants (135 HNSCC, 101 OSF, and 126 controls), Choudhary et al. studied the genotype of MMP-3 SNP by PCR-RFLP analysis. The difference in 5A genotype frequency between OSF and control was statistically significant. However, the 5A genotype showed a twofold increased risk for OSF development compared to controls, but this phenomenon was only observed in patients less than 45 years of age. The concluded that MMP-3 genotype expression is associated with 5A alleles, which may play a major role in developing HNSCC and OSF [36].

Zade et al. evaluated the genotype of MMP-3 SNP by PCR analysis of 20 individuals (five OSCC, five OSF, five normal individuals with AN and alcohol habits, and five without the habit). The frequency of 5A genotype in MMP-3 promoter was found to be higher in OSF compared to the control group; however, between OSCC and control group, no significant difference was noted. It was similar to Tu's study where 5A genotype in the MMP-3 promoter is associated with the risk of OSF but not in OSCC in an Indian population [37]. In MMP-3 promoter -1171, 5A>6A, the insertion or deletion of single adenosine could alter the transcription level of the MMP-3 gene. For MMP-3, the frequency of the 5A genotype in the MMP-3 promoter region was higher in the OSF group than in the control group and a greater than twofold risk for developing OSF compared to controls. However, the 5A/5A carrier allele showed an association only in patients less than 45 years of age [37].

9.3.6 TGF-β and SMAD

A variety of mediators including hormones, cytokines, and growth factors influence the synthesis of collagen TGF-β controls proliferation, differentiation, and function in many cell types. It is known to stimulate collagen production through the regulation of intramembrane proteolysis and CREB3L1 activation [38]. Kale et al. found more adipose tissues and TGF-β expression in early-stage OSF tissues in India [39]. Overexpression of both TGF-β1 and TGF-β2 was reported in OSF tissues, with a higher expression of TGF-β1 than TGF-β2. TGF-β1 is expressed in inflammatory cells, perivascular cells, epithelial cells, muscle and fibroblasts [40–42]. The mRNA level of TGF-β was higher in the early and middle stages of OSF tissues than in healthy patients of Hunan, China [42]. An increased expression of CD105, a TGF-β1 receptor, was associated with hypoxia-induced neoangiogenic activity in OSF. This feature was linked to transformation from normal mucosa to mild and severe epithelial dysplasia [43–45].

A study from Sri Lanka showed that the difference in TGF-β expression was not evident among OSF patients, pan masala (a chewing tobacco and AN product in India) chewers, and healthy oral mucosa. Secretion of TGF-β1 in cultured fibroblasts harvested from the specimens also showed no marked difference [46]. In a recent study, real-time PCR and immunohistochemical staining showed elevated expression of TGF-β1, connective tissue growth factor (CTGF), and decreased expression of bone morphogenetic protein 7 (BMP-7) in OSF. The possible stimulatory effects of areca components on epithelial-mesenchymal transition and expression of smooth muscle actin, CTGF, TGF-β, LIM domain kinase 1 (LIMK1), and p-Smad2 in epithelial cells has also been reported [47–49]. Injection of pan masala extract to buccal mucosa of Sprague Dawley rats on alternate days for 48 weeks induced OSF-like changes with a concomitant elevated expression of TGF-β1 [50].

Chang et al. observed that areca nut extract (ANE) stimulated TGF-β1 and Smad2 in oral keratinocytes and SAS oral cancer cells implicating the involvement of AN in the OSF pathogenesis [51]. Smad2 overexpression has been reported in OSF tissues relative to healthy tissues [52].

The expression of Smad7, an inhibitor of TGF-β, is elevated in OSCC and OSF tissues relative to normal tissues and has been suggested as a diagnostic marker [53]. The association of genotypes of TGF-β1 with the risk of OSF has been investigated. While comparing the frequencies of TC, TT, and CC alleles on the TGF-β1 gene on chromosome 19q, it was found that high OSF risk is associated with CC alleles in both low- and high-exposure groups [15]. An Indian study showed evidence of C-to-T transition (rs13306708) in the 50UTR region of TGF-β1 between OSF patients [27CC, 15CT, 8TT] and control subjects [42CC, 6CT, 2TT]); however, authors could not find polymorphisms in the promoter region and exon 1 of TGF-β1 in OSF patients and control subjects [54]. Hsu et al. compared the TGF-β1 codon 10 T/C and codon 25 G/C polymorphisms in patients with oral premalignant lesions and healthy control. They found an association of TGF-β1 codon 10 and 25 polymorphisms with the development of OPMDs [55].

9.3.7 Lysyl Oxidase (LOX)

Lysyl oxidase (LOX), also known as protein-lysine 6-oxidase, is a copper-dependent extracellular enzyme that mediates cross-linking of collagen and elastin through posttranslational oxidative deamination of peptidyl lysine residues in their precursors. LOX stabi-

lizes the collagen fibrillar array and contributes to the stiffness and mechanical strength of ECM [56, 57]. LOX and LOX-like proteins participate in tissue fibrosis, tumorigenesis, atherosclerosis, and metastasis by mediating protein expression and regulating signal transduction [56]. LOX overexpression affects tumor desmoplasia (fibrosis) and tumor microenvironment, and stimulates anchorage-independent growth of OSCC cells [56, 58, 59]. LOX expression in blood cells from patients of OSF was found to be similar to, lower than, or higher than age- and sex-matched controls suggesting that changes of LOX in circulating blood cells of OSF were not significant [60]. LOX activity is found to be elevated in fibroblasts cultured from OSF patients when compared with fibroblasts cultured from normal oral mucosa [61]. An epidemiological study showed elevated LOX expression in OSF and oral mucosa adjacent to OSCC tissues [58]. This could be due to the stimulation of LOX expression in oral keratinocytes by ANE. Copper in BQ was found to stimulate LOX expression of fibroblasts, thereby increasing collagen cross-linking and resistance to degradation [62].

LOX, encoded by the LOX gene on chromosome 5q, has been linked with predisposition to OSF. The frequencies of three genotypic variants (AA, AG, and GG) of LOX genes in patients of OSF and controls were investigated. The high OSF risk allele was found to be AA in the low-exposure group, while GG is more prevalent in the high-exposure group [15]. Differences of Arg158Gln SNPs of the LOX genotype between elder BQ chewers and OSF patients were seen in PCR-RFLP and direct sequencing [63]. The Arg158Gln SNP was associated with early clinical stages of OSCC [52]. Thorawat et al. studied LOX G473A SNP in OSF, in BQ chewers without OSF, as well as in healthy controls. LOX G473A SNPs were not present in this Indian cohort [64]. Mukherjee et al. studied the genotype for LOX polymorphism by PCR-RFLP. They observed significantly higher heterozygous G473A genotype in OSF cases, among 26–40-year age group, and in male patients. They also found a higher total soluble collagen level among patients carrying GA or AA genotype [65]. These reports suggest that LOX and LOX-like proteins could be a potential therapeutic target for the treatment of OSF [59].

9.3.8 Cystatin C (CST3)

Cystatin C (CST3) is a molecule responsible for the prevention of ECM degradation. The terminal regions of each collagen molecule are composed of terminal peptides, which function in cross-linking and enhancing the strength of collagen fibers. These areas are resistant to attacks by collagenases but are susceptible to serine and cysteine proteinases. These groups of enzymes belong to the cystatin superfamily, namely the type 1 cystatins (stefins A, B), type 2 cystatins, and kininogens. Cystatin C is one of the type 2 cystatins, a class of cysteine proteinase inhibitors found in a variety of human body fluids and secretions. The major function of this enzyme is to provide protection and stabilization of the collagen fibrils [66]. Cystatin plays a crucial role in carcinogenesis and fibrosis of various organs such as the kidney, lung, and liver [67–69]. Cystatin C expression is higher in OSF tissues when compared to normal oral mucosa. It is mainly expressed by fibroblasts, endothelial cells, and inflammatory cells. Fibroblasts from OSF show higher cystatin expression than normal fibroblasts. In addition, arecoline was found to promote cystatin C mRNA and protein expression in a dose-dependent manner [70]. Cystatin C is encoded by the CST3 gene on chromosome 20p. With A being normal allele and B the mutated allele, frequency distribution of AA, AB, and BB on CST3 gene in OSF patients and healthy individuals were showed an increased risk of OSF with high-risk allele AA in those with both low and high exposures to betel quid [15]. There was a consistent relationship between genotype distribution of TGF-β1 and CST3 genes and the risk of OSF in both low- and high-exposure groups [71].

The authors have also investigated the effect of the combination of these six genes for evaluating gene-gene interaction, in both high and low exposures for BQ. Other studies reported that genotypes associated with the highest OSF risk in the lower exposure group were CC of COL1A1, AA of COL1A2, TT of collagenase-1, CC of TGF-β1, AA of LOX, and AA of CST3. On the other hand, TT of COL1A1, BB of COL1A2, AA of collagenase-1, CC of TGF-β1, GG of LOX, and AA of CST3 genes led to the highest risk of OSF in the high-exposure group [71].

An elevated cystatin M was shown to promote the metastasis of OSCC by blocking cathepsin B activity and rescuing tumor cells from TNF-α-induced apoptosis [72]. Salivary cystatin B level was also found to be a valuable prognostic marker for OSCC patients [73]. More studies are needed to clarify the role of various cystatins in OSF and OSCC.

9.3.9 Plasminogen Activator Inhibitor (PAI-1)

ECM homeostasis and wound healing are regulated by plasminogen activator inhibitor-1 (PAI-1) by suppression of urokinase plasminogen activator (uPA)/tissue plasminogen activator (tPA)-mediated conver-

sion of plasminogen to plasmin, which activates MMPs and fibrinolysis. Studies on fibrosis models of internal organs such as liver, lung, and kidney have found that PAI-1 deficiency or inhibition of PAI-1 activity attenuates organ fibrosis [74]. TGF-β may stimulate PAI-1 expression through ROS and SMAD-dependent (ALK5/smad2/3) and SMAD-independent (Src/EGFR/MEK/ERK) pathways [75, 76]. When compared to normal buccal fibroblasts, PAI-1 and tPA secretion is increased in fibroblasts derived from OSF. In OSF, the fibroblast ratio of PAI-1/tPA is also increased. Arecoline stimulates PAI and tPA secretion. It also increases the PAI-1/tPA ratio in buccal mucosal fibroblasts [77, 78]. Moreover, when compared to healthy tissue, hypoxia-inducible factor-1α (HIF-1α) showed overexpression in fibroblasts, inflammatory cells, and epithelial cells in OSF tissues. Hypoxia enhanced arecoline-induced ECM and PAI-1 production by buccal mucosal fibroblasts [79]. PAI-1 expression was found to be elevated in OSCC tissues when compared to normal tissues; however, PAI-1 showed little association with the survival rate of OSCC patients [80]. PAI-1 – 675 4G/5G genotypes were reported to be strongly associated with OSCC relative to control subjects in the European population [81, 82]. More studies can clarify PAI-1 polymorphism in the risk and survival of OSF and OSCC patients.

9.3.10 TIMPs (Tissue Inhibitor Matrix Metalloproteinases)

TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) that show differential inhibitory effects on MMPs, a disintegrin, and metalloproteinases (ADAMs) prevent proteolysis of ECM followed by accumulation of ECM/tissue fibrosis [16]. Shrestha and Carnelio reported overexpression of MMP-2 and TIMP-2 in mucosal tissues from early and moderately advanced OSF patients. TIMP-2 was expressed in the deeper connective tissue, lamina propria, and suprabasal layers. MMP-2 found in the basal and suprabasal layers showed differences in expression in these two stages of OSF [83]. Zymography and immunohistochemical staining showed an increase in the expression of MMP-1, MMP-2, MMP-9, TIMP-1, and TIMP-2 in OSF tissues when compared to normal tissues [23]. An in vitro study from Sri Lanka found that fibroblasts from early-stage OSF secrete more TIMP-1 and TIMP-2 compared to fibroblasts from pan masala chewers without OSF and normal buccal mucosal fibroblasts. These differences might be caused by cellular senescence and premature aging of buccal mucosa tissues [46].

Safrole and arecoline stimulate TIMP-1 mRNA and protein expression of buccal fibroblasts [32, 84]. Fibroblasts from OSF tissues were found to secrete more TIMP-1 than fibroblasts from surrounding healthy tissues suggesting that they may have role in the pathogenesis of OSF [32, 84].

Fibroblasts from early-stage OSF were found to have similar levels of TGF-β1, MMP-1, MMP-2, MMP-3, interleukin-6 (IL-6), and IL-8 when compared with fibroblasts from healthy tissues. However, increased TIMP-1 and TIMP-2 secretion from OSF fibroblasts was observed [46].

9.3.11 Vascular Endothelial Growth Factor (VEGF)

Transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) are tumor growth factors that serve as cancer biomarkers [85]. Adequate blood supply is necessary for the growth of solid tumors, and growth factors responsible for it have been studied extensively in various tumors [86]. Vascular endothelial cells are found to be dormant in adult cells, and they divide less than once in a decade. During the tumor growth phase, hypoxia develops, pro-angiogenic factors increase resulting in angiogenesis and tumor progression. Following upregulation of hypoxia-inducible transcription factor, increased production of VEGF is observed [87]. VEGF is a critical angiogenic cytokine involved in blood supply to neoplastic cells. Also, there is a significant increase in vascularity during the transition from normal oral mucosa to degrees of dysplasia. In addition to its role in angiogenesis in squamous cell carcinoma, VEGF has also been investigated in oral potentially malignant disorders and has been linked to oral cancer metastasis [88]. The polymorphic nature of the VEGF-460/T gene has been assessed in subjects of OSF to identify the progression to malignancy at an early stage [89]. Among subjects, 6.67% showed CT polymorphism and 16.67% of subjects showed TT polymorphism. Thus, VEGF 460 C/T has the potential to be used as a prognostic marker in predicting the malignant transformation of OSF [89].

9.3.12 Cytochrome P450 (CYP3A) Gene

Cytochrome P450 3A (CYP3A) gene family has a major role to play in the oxidative metabolism of many xenobiotic substrates and active endogenous substrates. Polymorphisms in the gene for cytochrome P450 enzyme can alter the activation or detoxification of carcinogenic

compounds that influence an individual's genetic susceptibility to cancer [90]. Betel chewers having lower CYP3A expression levels are more susceptible to BQ toxicity. Various studies have demonstrated CYP3A gene polymorphism associated with an individual's susceptibility to OSCC and OPMDs among tobacco users [91, 92].

CYP3A polymorphism had also been identified as a genetic marker for OSF susceptibility [93–96]. Choudhari et al. suggested polymorphism in CYP1A1 and CYP2E1 was associated with an increased risk of OSF [94]. Yaming et al. reported a significant association between the gene polymorphism of CYP1A1 at the NcoI site and the risk of OSF when compared to controls. However, no such association was observed for the CYP2E1 gene polymorphism in OSF patients [95]. Identifying CYP polymorphism can be used to screen individuals who are genetically at high risk of developing OSF and cancer, thus helping in guiding treatment regimens for patients.

9.3.13 DNA Repair Gene Polymorphism

9.3.13.1 X-Ray Cross-Complementing (XRCC) Polymorphism

X-ray cross-complementing (XRCC) genes were discovered mainly through their role in protecting mammalian cells from damage caused by ionizing radiation. They are also important in genetic stability. There are two main pathways in eukaryotic cells for repairing double-strand DNA breakage, namely, nonhomologous end joining and homologous recombination (HR). Nonhomologous end joining provides a mechanism for the repair of double-strand DNA breakage throughout the cell cycle. However, it is particularly important during the G0, G1, and early S phases in mitotic cells and is mediated by the XRCC5, XRCC6, and XRCC7 genes. The DNA repair protein Ku acts as a heterodimer of the two 70 kDa (Ku70) and 80 kDa (Ku80) subunits and binds to DNA ends, nicks, or single- to double-strand transition. It serves as a DNA-binding component of the DNA-dependent protein kinase (DNA-PK) that phosphorylates certain chromatin-bound proteins in vitro. The XRCC5 gene encodes Ku80 and forms a heterodimer with Ku and functions as DNA end binding at the double-strand breakage site. Ku binds to the end of the DNA double-strand breakage and appears to stabilize the binding of the DNA-PKCs to DNA [97–100].

9.3.13.2 NADPH Quinone Oxidoreductase 1 (NQ01) C609T

The human NAD(P)H:quinone oxidoreductase 1 gene (NQO1; DT-diaphorase, Enzyme Commission (EC) number 1.6.99.2) occupies 17 kilobase pairs (kb) within a gene-rich region on chromosome 16 at 16q22.1 [101]. This cytosolic flavoenzyme detoxifies quinones (a large class of aromatic compounds found commonly in plants, benzene metabolites, and chemotherapies) to hydroquinones or catechols. The enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) acts as an antioxidant by catalyzing a 2-electron reduction that bypasses the need for two 1-electron reductions that can result in the production of DNA and protein-damaging reactive oxygen species. In certain conditions (e.g., the presence of

myeloperoxidase or antioxidants), NQO1 can contribute to the formation of reactive oxidation species via oxidative cycling and therefore can act as a prooxidant [102]. NQO1 is constitutively expressed in most tissues including the bone marrow, where expression is thought to be highly inducible by xenobiotics with quinone moieties and is upregulated during times of oxidative or electrophilic stress. Polymorphisms of NQO1 gene have been characterized and known for about two decades [103]. C-to-T substitution at position 609 of NQO1 cDNA codes for a proline-to-serine change at residue 187 is now documented in most cancers.

9.3.14 Tumor Necrosis Factor-α (TNF-α)

Tumor necrosis factor-α (TNF-α), situated in the class III region of human leukocyte antigen (HLA), is a mediator with multiple functions, including the regulation of inflammation and transcriptions of collagen and collagenase. Chiu et al. studied a biallelic promoter-region (-308) polymorphism on the TNF-**α** gene and showed a significantly lower TNF-2 allele among OSF subjects than in areca-chewing controls, implying a multifunctional etiological factor of TNF-**α** in OSF pathogenesis [104].

9.3.15 p53 Gene Mutations

The p53 gene is a tumor-suppressor gene that is found in the mutated form in common human cancers including OSCC. Characteristics of mutations in the p53 gene of OSCC in betel quid chewers in Sri Lanka have been reported [105].

Trivedy et al. carried out mutation analysis of exons 2–9 for studying p53 mutation in OSF and OSCC by examining the mobility shift in single-strand conformation polymorphism (SSCP). They observed mobility shifts in SSCP indicative of p53 mutation or loss of heterozygosity (detection of the band) in OSF (13/21) and OSCC (15/27), suggesting that p53 mutation/protein stabilization plays a possible role in the pathogenesis of OSF and its malignant transformation [106].

9.3.16 Cytotoxic T-lymphocyte-Associated Antigen 4 (CTLA-4); CD 152 (Cluster of Differentiation 152) Gene Polymorphism

CTLA-4 is a negative regulator of T-lymphocyte activation. The particular genotype of the locus encoding the CTLA-4 glycoprotein has been associated with susceptibility to various autoimmune diseases. Shin et al. investigated the possible role of CTLA-4 polymorphism in OSF susceptibility by restriction fragment length polymorphism (RFLP). They found that the G allele at position +49 of exon 1 was significantly associated with OSF, confirming its role in the risk for the development of OSF [107].

9.3.17 Major Histocompatibility Complex (MHC) Class I Chain-Related Gene A (MICA) Polymorphism

MICA is expressed by keratinocytes and other epithelial cells, and its encoded protein interacts with ϒ/δ T-cells localized in the submucosa. Liu et al. analyzed MICA polymorphism in OSF using the ABI prism 377-18 DNA sequencer. The phenotype frequency of allele A6 of MICA in OSF was significantly higher than that of controls, suggesting that allele A6 in MICA might confer risk for OSF [108].

9.3.18 Glutathione S-Transferase (GST) Polymorphism

Several allelic variants of polymorphic glutathione S-transferases (GSTs) show impaired enzyme activity and are suspected to increase the host's susceptibility to various cancers. Agrawal et al. showed a higher frequency of both the GSTM1 and GSTT1 null genotype in OSF cases than in controls, suggesting a greater risk associated with the genotype in the development of OSF [109].

9.3.19 Apoptosis-Associated Genes FAS and FASL Polymorphism

Wang et al. demonstrated that FAS polymorphism in the form of FAS A_{-1377} G_{-670} versus FAS G_{-1377} $-A_{-670}$ haplotype is significantly correlated with the malignant potential of OSF [110].

9.3.20 Loss of Heterozygosity (LOH)

Teh et al. provided evidence of genomic instability in the form of loss of heterozygosity (LOH) in OSF. This acquisition of LOH may subsequently alter gene function and expression. Nearly 50% of OSF cases included in this study demonstrated a small number of discrete hot-spot LOH loci, and the degree of LOH showed a significant positive relationship with OSF grade. Chromosome 13 contains the largest LOH regions from 13q14 to 13q33, in proportion to chromosomal size. Since the chromosome 13q is highly susceptible to genomic instability in HNSCC, it was hypothesized that genes within the 13q14–q33 LOH region found in the OSF may have a crucial part in the initiation of oral carcinogenesis in these patients. Along with this, few other LOH loci with previously identified susceptibility regions in HNSCC: 3p24-p22, 6q26-q27, 9q22.3, 12p11.2, and 20p12-11, were recognized in this study [111].

9.4 Conclusion

OSF is an oral potentially malignant condition with a potential for malignant transformation. The pathophysiology of OSF is very complex. Areca nut chewing is the etiological factor in the causation of OSF. Though certain individuals due to their genetic constitution are less prone to develop the disease, susceptible individuals may develop the disease over a short period of exposure to areca nut [112]. The genetic alterations in OSF influence TGF-β pathway and collagen metabolism, inflammatory response, immune response, epithelial-mesenchymal transition, and epithelial differentiation/keratinization. It is, therefore, important to further clarify the molecular mechanisms of BQ-induced OSF and oral cancer to identify high-risk individuals.

Summary

BQ components are found to induce extracellular matrix (ECM) deposition via upregulation of TGF-β1, PAI-1, cystatin, LOX, TIMPs, MMPs, collagenase-1, SMAD, VEGF, cytochrome P450, XRCC, NQO1, TNF-α, P53, CTLA-4, CD, MICA, GSTs, FAS, and FASL. The high-risk alleles and genotypes of collagen, TGF-β1, PAI-1, cystatin, LOX, TIMPs, MMPs, collagenase-1, SMAD, VEGF, cytochrome P450, XRCC, NQO1, TNF-α, P53, CTLA-4, CD, MICA, GSTs, FAS, and FASL found in OSF patients with high frequency may change the transcriptional activity and the functions of corresponding proteins and increase the risk of OSF.

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