Review

A potential protection of melatonin on pathogenesis of oral sub-mucous fibrosis (OSMF) : a current update

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ABSTRACT

Oral submucous fibrosisis (OSMF) is a clinical condition of the oral cavity which is caused predominantly by areca nut consumption. This fibrotic condition affects almost all parts of the oral cavity and can cause significant reduction in mouth opening, thereby, resulting in functional impairment. The other potential risk of OSMF is its malignant transformation into oral squamous cell carcinoma, which occurs in a significant number of afflicted patients. Extensive researches have been conducted to understand the pathogenesis of OSMF for identification of tangible therapeutic modalities. To date, there is no effective therapeutic modality for this disorder. It is well known that melatonin has a potent anti-fibrotic, anti-oxidant, and pro-angiogenic effects. The therapeutic potential of melatonin on OSM cannot be ignored. In this article we have explored the potential mechanisms of melatonin as an adjuvant in the prevention and treatment of OSMF.

Key words: Antifibrotic, antioxidant, immunomodulation, melatonin, oral submucous fibrosis

1. INTRODUCTION

Oral submucous fibrosis (OSMF) was first clinically identified in individuals of Asian descent as a oral cavity disease with the potentiality of malignant transformation (1). This is an insidiously chronic disease that affects any part of the oral cavity and sometimes even the pharynx. It has several nomenclatures including idiopathic scleroderma of mouth, idiopathic palatal fibrosis, sclerosing stomatitis and juxta-epithelial fibrosis (1). The hallmark of this

disease is the widely spreaded fibrosis of the oral mucosal tissue, which causes progressive trismus due to rigid lips at cheeks and dysphagia due to fibrosis of the upper third of the esophagus (2). The disease is mainly encountered in the Asian subcontinent and the prevalence is higher in India than other countries (2). OSMF was originally reported by Schwartz in 1952 when he examined five Indian women from Kenya. He initially coined the term "atrophic aidiopathica (tropica) mucosae oris" (3). Subsequently, in 1953, Joshi, another clinician from Mumbai renamed this disorder as OSMF (4). It was also considered by some clinicians as a collagen disorder of the oral tissues in the last decade (5, 6). The commonly accepted definition of OSMF is that it is an insidious chronic disease affecting any part of the oral cavity and sometimes pharynx, although, occasionally preceded by and/or associated with vesicle formation and always associated with juxta-epithelial inflammatory reaction followed by fibroblastic changes in the lamina propria with epithelial atrophy leading to stiffness of the oral mucosa causing trismus and difficulty in eating (7).

A large proportion of patients have difficulty to consume spicy food, mouth toughness, lack of laxity of lip, tongue, and palate leading to difficulty in mouth opening. The disease is prevalent in countries where individuals have betel chewing habit. Arecoline present in areca nut has been confirmed to be the principal factor in causing this disease (1). The habit of betel quid chewing is characterized by the consumption of piper betel vine leaf- wraps in which fragments of areca nut, slaked lime, and tobacco are packed. During this process arecoline is released from the areca nut to initiate OSMF. However, multiple factors also promote the etiopathogenesis of OSMF and they are discussed below.

2. PATHOGENESIS OF OSMF

2.1. Areca nut consumpation induced inflammation and role of inflammatory cytokines and enzymes.

Areca nut chewing initially causes an acute inflammatory reaction that can be aggravated by co-consumption of slaked lime. Interaction of areca nut contained components with the polymorphonuclear cells (PMCs) causes increased production of reactive oxygen species (ROS) (8). ROS, then, increases the nuclear factor kappa B (NF*k*B) expression which, in turn, up-regulates pro-inflammatory cytokines such as interleukin 1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and proinflammatory enzyme, cyclooxygenases leading to juxta-epithelial fibrosis (9, 10). The sustained inflammatory response and the alterred collagen and collagenase production caused by TNF-α play the significant roles in OSM pathogenesis (11). Genetic studies have demonstrated that individuals with homozygous wild genotype TNF-α2 have the increased risk of OSMF and the mutant allele TNF-α2 has 7 times greater intensity in enhancing promoter function in comparison with wild allele. Hence, TNF-α could be considered to play a pivotal role in OSMF pathogenesis (11). The cyclooxygenase 2 (COX2) is another important factor influenced by arecoline. An immunohistochemical study on OSMF tissue showed the upregulated expression of COX2 compared to control, highlighting the relationship between cyclooxygenase and the pathogenesis of OSMF (12). This has also been observed in an *in vitro* study of oral keratinocytes in which arecoline treatment was found to upregulate COX2 expression and prostaglandin production (13).

2.2. Role of oxidative stress and cell cycle related proteins.

Ample evidence points out the role of oxidative stress in OSMF. An increased level of biomarkers related to oxidative stress and depleted antioxidant status are often observed in OSMF(14, 15). Oxidative stress damages macromolecules including lipids, proteins and DNA. For example, arecoline induces DNA damage. Human keratinocytes treated with areca nut extracts significantly increases the level of 8 hydroxydeoxy guanosine, thus, highlighting the arecoline induced oxidative DNA damage (16). With regard to the genotoxicity, arecoline also causes cell cycle disruption. In an *in vitro* study, arecoline administration inhibits epithelial cell proliferation and survival by inhibition of G_1/S phase regulatory proteins cyclin D1, (appears in the G1 phase of the cell cycle) CDK4, CDK2 and E2F1 (expressed in the late G1 to S transition phase). The mentioned mechanisms above have a significant impact on epithelial atrophy observed in OSMF lesions (17).

2.3. Role of immune cells.

The immune cells also involved in the OSMF pathogenesis including mast cells and Langerhans cells (LCs). The LCs are dendritic, non-keratinocyte clear cells located in the supra-basal layer of the oral mucosal epithelium and are the well established antigen-presenting cells (APCs). An immunohistochemical study has demonstrated the increased numbers of LCs in oral tissue of patients with OSMF compared to healthy individuals (18). It suggests that LCs may recognize areca nut constituents as foreign antigens through MHC class 2 and present these antigens to lymphocytes to trigger a specific immune response. Histopathological studies have also reported an increased density of mast cells in oral submucous fibrosis (19), suggesting their role in cytokine production, especially, the transforming growth factor-alpha (TNF-α) that may accentuate fibrosis.

2.4. Role of transcription factors and growth factors.

Concerning the role of transcription factors other than NF*k*B in OSMF pathogenesis, SMAD-2 (mothers against decapentaplegic homolog 2) deserves special attention. Epithelial cells treated with catechin, tannin, and alkaloids have higher level of SMAD-2 phosphorylation than that of the untreated controls (20). In addition, ALK5, JNK, and p38 MAPK pathways also participate in the pathobiology of OSMF (21, 22). The signaling pathways mentioned above culminate in the increased expression of growth factors such as TGF-beta, IGF-1, b-FGF, and CTGF. It has been reported that arecoline promotes production of alpha 5 - beta 6 (α5-β6) integrin which, in turn, upregulates TGF-β expression in oral tissues (23). TGF-β is a key molecule involved in OSMF pathogenesis and its signaling as the main predisposing factor for synthesis of collagen in OSMF has been elucidated by the global gene expression profiles induced by TGF-β in epithelial cells isolated from the oral cavity (20). Oral epithelial cells exposed to aqueous extract of areca nut containing polyphenols and alkaloids share 64% similarity with those treated by TGF-β, in regard to their gene expression patterns (20). It is understandable since arecoline causes induction of TGF-β expression in oral epithelial cells, this finally leads to the onset of tissue fibrosis. Indeed,TGF-β predominates during the early stages of OSMF and becomes less abundant with the progression of the condition (20). Studies conducted on OSMF patients revealed an upregulated b-FGF expression in the fibroblasts during the early phase of

inflammation. This indicates that bFGF is associated with the initial injury caused by the exposure to arecoline. bFGF further stimulates the release of other pro-inflammatory cytokines that exacerbates fibrosis (24). The b-FGF also increases during the progression of the OSMF and it is strongly expressed in the stromal cells as the disease progresses (24). The expression of bFGF, however, declines in the fibroblasts and endothelial cells with the progress of OSMF (24). A significant up-regulation of insulin growth factor-1 (IGF-1) expression at levels of mRNA and protein in OSMF has been reported and attributed to arecoline in a dose-dependent manner (25). The induction and further progression of fibrosis in human oral tissues are also associated with connective tissue growth factor (CTGF) which increases level in OSMF compared to healthy oral tissues, at the onset and during the advanced stages of fibrosis (26).

2.5. Role of MMP and heat shock proteins.

OSMF is also termed as a collagen disorder (5, 6). In light with this, the role of matrix metalloproteinases (MMPs) needs to be addressed. These zinc-dependent metalloproteinases degrade collagen while tissue inhibitors of matrix metalloproteinases (TIMPs) are found to inhibit collagen degradation. It has been found that the imbalance between MMPs and TIMPs in OSMF occurs with the reduced expression of MMP1. In one hand, MMP1 degrades fibrillary collagen, on the other hand, it significantly increases the expression of TIMP, as a result, to prevent collagen degradation (27). The net result is to increase collagen accumulation causing exacerbated extracellular matrix deposition. It has also been found that the heat shock proteins (HSPs) which are involved in pro-collagen secretion are also over-expressed in OSMF. The increased HSP47 expression in OSMF at levels of the mRNA and protein has been reported (28). The increased HSP47 expression coupled with increased malondialdehyde (MDA) production are attributed to increased collagen cross-linking in OSMF (29).

2.6. Role of copper.

Another important enzyme, involved in collagen cross-linking and extracellular matrix organization, is lysyl oxidase. This enzyme is a copper-dependant enzyme. It is noteworthy that areca nut extracts are copper-rich and hence elevates copper levels in saliva in habitual chewers (30). Consequently, the copper ions are absorbed into the buccal mucosa and increase lysyl oxidase activity thereby leading to increased collagen cross-linking and extracellular matrix components in OSMF(30).

2.7. Role of autoimmune activity.

An autoimmune basis in OSMF pathogenesis has been proposed (9). The histologic resemblance of the oral submucous fibrosis lesions with scleroderma, an autoimmune disorder, has shed light on a possible role of autoimmunity in the pathogenesis of OSMF. The expression of CCL2 as a common marker in both scleroderma and oral submucous fibrosis has been reported. Autoantibodies against the antinuclear antigen, smooth muscle antigen, gastric parietal cell antigen and thyroid microsomal antigens in patients with oral submucous fibrosis have been reported (9). Increased levels of salivary and serum IgA, IgG levels in oral submucous fibrosis patients further support the concept of autoimmunity in OSMF pathogenesis (31).

2.8. Role of renin angiotensin system and endothelin.

It is well known that a tissue renin-angiotensin system exists in the oral tissue (32). Angiotensin2, the effector peptide of the system, causes profibrotic action in oral fibroblasts mediated through the receptor AT-1(33). One of the important tissue convertors of Angiotensin 1 to 2 is mast cell chymase (34). Notably, mast cells are accumulated in OSMF (19). Hence, the increased chymase level will cause overexpression of Angiotensin2 to mediate profibrotic activity. Another significant molecule implicated in fibrosis is endothelin1 which is a 21 amino acid-containing peptide and it also has profibrotic activity (35). A clinical study has implicated higher endothelin 1 level in saliva samples of patients with oral submucous fibrosis compared to healthy subjects (36).

2.9. Role of epithelial mesenchymal transition.

Epithelial-mesenchymal transition (EMT) is another important phenomenon that has been implicated in OSMF (37). This mechanism functions in both physiological and pathological situations. EMT denotes the phenotypic conversion of epithelial cells into myofibroblast-like cells after the loss and gain of certain molecular markers. In connection with OSMF, EMT may play a major role in this disorder. Cell injury caused by Areca Nut Extracts (ANE) produces aberrant amounts of ROS which in turn triggers both MAPK and NF-κB pathways involved in EMT (37). Furthermore, the upregulated expression of TGF beta in OSMF is sufficient to explain the basis of EMT as TGF beta is a key molecule in triggering EMT. Epithelial-mesenchymal transition as an event predisposing to OSMF is also supported by the presence of myofibroblast-like cells in OSMF tissues (38).

2.10. Malignant transformation of OSMF.

OSMF as a potential premalignant disorder significantly increases the rate of malignant transformation. Areca nut is a carcinogen with cytotoxic and genotoxic properties due to its component arecoline (39). The presence of high copper content in areca nut also is an important issue of concern since copper levels in saliva are elevated in oral cancer patients (30). The induction of oxidative stress with the consequential generation of ROS and other toxicity species along with aberrant inflammation by areca nut extracts could also predispose to malignant transformation of OSMF. The up-regulation of proliferation markers like PCNA (40) and Ki 67 (41) in OSMF demonstrate an inclination of this lesion towards malignant transformation. Another important molecule, hypoxia-inducible factor1 (HIF1), is also over-expressed in OSMF. HIF1 plays a critical role in the malignant transformation of OSMF lesions (42).

3. TREATMENT MODALITIES FOR OSMF

Considering the morbidity and malignant transformation of OSMF it is important to establish the effective treatments for this disorder. A plethora of treatment options have been tested practically and threotically. These include the use of antioxidants (43), herbal extracts with antifibrotic activity (44), intralesional steroid and enzyme injections (45), a few to mention. Surgical excision of the fibrotic bands that result in restricted mouth opening have also been implemented (46). Specifically to antioxidant therapy as an adjuvant in OSMF management,

several antioxidants have been tested. Out of these antioxidants, lycopene deserves attention. Lycopene is initially suggested as a potential antioxidant in the management of OSMF (47). Clinical study has proven the long term efficacy of lycopene in the treatment of OSMF (48). Lycopene is a structurally symmetrical tetraterpene consisting of 8 component isoprene units, comprising 11 conjugated and 2 non-conjugated double bonds between the component carbon atoms (49). It is a member of the carotenoid family and is an important phytoconstituent of tomato. Lycopene detoxifies ROS including singlet oxygen (50) and hypochlorous acid (51). Compared to lycopene, melatonin is a more potent antioxidant with anti-inflammatory and immunoregulatory activity (52, 53). Thus, we hypothesize that melatonin may exhibit beneficial effects in in the management of OSMF. The mechanisms will be discussed below.

4. MELATONIN: A BRIEF INSIGHT

Melatonin is a low molecular weight indoleamine produced and secreted principally by the pinealocytes of pineal gland in vertebrate (54). A complex biochemical pathway underlies the biosynthesis of melatonin from its precursor tryptophan. The enzymes that are involved in the biosynthetic pathway are tryptophan-5-hydroxylase, 5-hydroxytryptophan decarboxylase, Arylalkylamine N-acetyltransferase (AANAT), and hydroxy indole-O-methyltransferase (HIOMT, currently the ASMT) (55). Melatonin is also synthesized in extra-pineal sites (56). In the oral cavity, the salivary glands (57) and the gingival tissues (58) are documented sites of melatonin production. The receptors of melatonin are also present in the oral cavity and in the gingiva (58). It has been well documented that melatonin is a potent antioxidant (52) and an anti-inflammatory agent (53) apart from its function as a regulator of circadian rhythm (59). The antioxidant potential of melatonin is superior to conventional antioxidants such as vitamin A, E and C. It is to be emphasized that melatonin can protect cells against oxidative damage more efficiently than other antioxidants under *in vivo* conditions (60). Melatonin and its secondary and tertiary metabolites are endowed with the potential to neutralize numerous toxic oxygen metabolites. By this mechanism, one melatonin molecule can scavenge up to ten ROS versus many classic antioxidants that scavenge one. The products (or metabolites) of melatonin interaction with ROS and reactive nitrogen species (RNS) retain their capacity to continue scavenging free radicals. Several studies have reported that the melatonin metabolite, cyclic-3-hydroxymelatonin, is more efficient than melatonin to scavenge the hydroxyl radical and other ROS (61). This is also the matter of fact for its tertiary metabolites, AMK and AFMK (62). With regard to its anti-inflammatory and immunomodulatory activity, melatonin inhibits activation of NF-kappa B, retards LPS-stimulated TNF-α, IL-1β, IL-6, IL-8 and IL-10 production in Raw264.7 cells through a mechanism involving downregulation of NF-κB activation (63). Melatonin has also been found to inhibit LPS-induced COX-2 and inducible nitric oxide synthase (iNOS) protein levels in the murine macrophage cell line Raw264.7 (64). Given the antixodant, antiinflammatory and immunomodulatory role of melatonin, it could be considered a key player in the treatment of many human diseases.

5. THE HYPOTHESIS

We hypothesize that melatonin could play a very significant role in the alleviation of the pathogenesis of OSMF. In addition to its activity mentioned above, melatonin is also a potent anti-fibrotic molecule (65). The beneficial effects of melatonin in ameliorating fibrosis have been

extensively documented (66). An extensive literature search has demonstrated that melatonin intervenes in many mechanisms that promote the pathogenesis of OSMF. This has been corroborated from the evidence of melatonin actions in other models of fibrosis. These data are summarized in Table 1. To help better understanding of the potential mechanisms of melatonin as an antifibrotic molecule in the context of OSMF pathogenesis are illustrated in figure 1.

Table 1: Summary of evidence for the therapeutic potential of melatonin in fibrotic conditions.

Distinct events in the pathogenesis of OSMF	**Evidence of melatonin's protection against fibrosis in variety of tissues**
Oxidative stress and depleted antioxidants	Reduction in the level of malondialdehyde in a rat model of carbon tetrachloride-induced liver fibrosis (67). Reduction in malondialdehyde levels and increases in the levels of glutathione and superoxide dismutase in a dimethylnitrosamine induced liver fibrosis in rats (68).
Increased levels of pro-inflammatory cytokines like IL 1 beta and TNF alpha	Reduction in the levels of IL-1β, TNF-α, and IL-6 in thioacetamide-induced liver fibrosis in rats(69).
Increased levels of arecoline mediated COX-2 production	Reduction in COX-2expression in bleomycin induced lung fibrosis model in mice (65).
Increased levels of oxidative DNA and production of 8 hydroxy deoxyguanosine	Reduction in 8 hydroxy deoxyguanosine levels in gray and white matter of mice subjected to focal cerebral ischemia (70).
Increase and dysregulation in numbers of Langerhans cells and mast cells	Reduction of mast cell degranulation in the dermis of the rat model upon injection thereby preventing the release of mast cell granule contents (71). Normalization of the circadian rhythm and controlled Langerhans and dendritic cell trafficking in blood and skin(72).
Increased expression of NF kappa B	Reduction in the expression of NF kappa B in a rat model of carbon tetrachloride-induced liver fibrosis (67).
Increased expression of SMAD, MAPK, JNK, P38	Reduction of SMAD expression in carbon tetrachloride-induced hepatic fibrosis (73)in rats. Reduction of MAPK expression of renal injury and fibrosis (74) and JNK and p38 expression in carbon tetrachloride-induced hepatic fibrosis model in rats (75).
Increased expression of TGF beta	Attenuation of TGF beta expression in models of hepatic and renal fibrosis by inhibiting SMAD, MAPK, JNK, and p38 expression (73-75).
Increased expression of b FGF	Reducion in the expression of TGF beta and b FGF in a nerve anastomoses model thereby reducing scarring and fibrosis at the nerve ends of pinealectomized animals (76).
Increased expression of CTGF	Significant reduction in the expression of CTGF in carbon tetrachloride-induced hepatic fibrosis model in mice (77).
Reduction in MMP levels and increased TIMP levels	Significantly lowering the levels of MMP9 and TIMP1 in carbon tetrachloride-induced hepatic fibrosis in mice (78).

Increased levels of copper	Mitigation of copper-induced oxidative damage in rat liver homogenates (79). Management of Wilson's disease due to its copper chelating properties (80).
Autoimmunity phenomenon	Clinical improvement in multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis through effects on immunoenhancement and inhibition of autoantibody production (81).
Increased expression of Angiotensin 2	Amelioration of chronic kidney damage and fibrosis induced by Angiotensin 2 (82).
Increased expression of endothelin 1	Reducionof synthesis and expression of endothelin 1 in a colon cancer cell line predominantly through NF kappa B inhibition (83).
Role of EMT	Inhibition of EMT induced by TGF beta 1 in lung alveolar epithelial cells (84).
Up-regulation of proliferation markers such as PCNA and Ki 67 implicating malignant transformation	Reduction in PCNA and Ki 67 expression thereby exerting antiproliferative effects in prostate cancer cell lines (85).
Up-regulation of hypoxia-inducible factor 1 alpha (HIF-1α) implicating malignant transformation	Inhibition of tumor angiogenesis in colon cancer cell lines by downregulating HIF-1α expression (86).

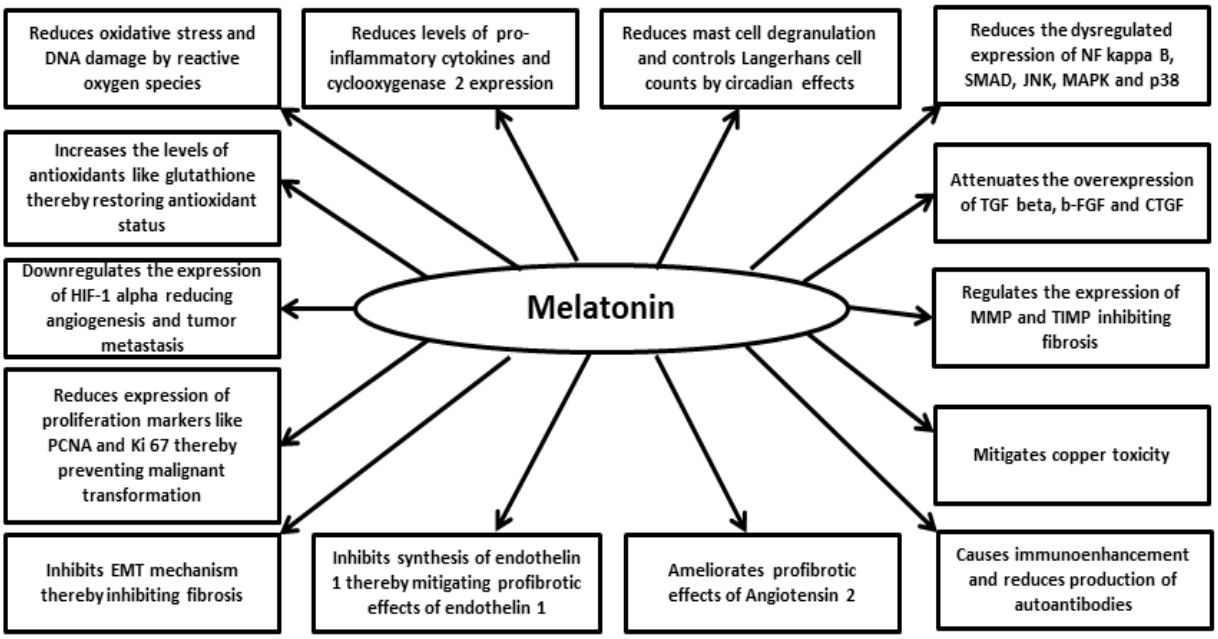

Figure 1: The potentially protective mechanisms of melatonin on OSMF pathogenesis.

6. CONCLUSION

Data from the table 1 and illustration from figure 1 summarize the potential mechanisms of melatonin to retard fibrotic formation in variety of organs and tissues. These include its effect on oxidative stress, inflammation, and immune regulation (69). Melatonin modulates enzymes related to inflammation such as COX-2 (65) and related to matrix remodelling such as matrix metalloproteinases and their tissue inhibitors (78). Moreover, melatonin is a potent modulator of transcription factors including SMAD, MAPK, JNK, p38 (73, 74). Through modulation of downstream signalling pathways of these transcription factors melatonin suppresses activities of growth factors including TGF beta, b FGF and CTGF. These growth factors promote OSMF pathogenesis (75-77). Melatonin can chelate transition metal, copper, to reduce its toxicity and mitigates its pivotal role in OSMF pathogenesis (79, 80). With regard to the autoimmune activity which predispose to OSMF, melatonin can suppress this activity as it does in many other autoimmune conditions (81). Melatonin can target renin angiotensin system and endothelin and its receptors which play a key role in OSMF pathogenesis (82, 83). OSMF favors the malignant transformation. Melatonin inhibits epithelial-mesenchymal transition phenomenon to provent this malignant conversion of OSMF (84). Morover, melatonin inhibits the signaling pathway of HIF-1α to prevent malignant transformation of lesions (86) and, thus, lowers the the malignant transformation rate of OSMF (85). All these provide compelling evidence for a potential therapeutic role of melatonin in OSMF. It is well established that salivary glands and gingival tissues can synthesize melatonin (58), which in turn is secreted into saliva. However, with increased inflammation and oxidative stress induced by areca nut chewing, the endogenous melatonin levels could be depleted thereby eliminating its protective effects. In this regard, using melatonin locally in the form of lozenges, gummies, mouth-washes, gel, and ora-base could potentially be of significant effect in the prevention and adjuvant management of OSMF.

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AUTHORSHIP

TMB, SV and RJ contributed to conception of the hypothesis and prepared the initial draft of the manuscript. DB supervised the work and critically revised the manuscript. SP and ATR contributed in editing and formatting the final manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Rajendran R (1994) Oral submucous fibrosis: Etiology, pathogenesis, and future research. *Bull. World Health Organ* **72**: 985–996.
2. Gupta MK, Mhaske S (2008) Oral submucous fibrosis: Current concepts in etioathogenesis. *People's J. Sci. Res.* **1**: 39-44.

3. Schwartz (1952) Atrophia idiopathica mucosa oris. In: Demonstrated at the 11th International Dental Congress
4. Joshi S (1953) Fibrosis of the e palate and pillars. *Ind. J. Otolaryngol.* **4:** 1-4.
5. Utsunomiya H, Tilakaratne WM, Oshiro K, Maruyama S, Suzuki M, Ida-Yonemochi H, Cheng J, Saku T (2005) Extracellular matrix remodeling in oral submucous fibrosis: its stage-specific modes revealed by immunohistochemistry and in situ hybridization. *J. Oral. Pathol. Med.* **34**: 498-507. https://doi.org/10.1111/j.1600-0714.2005.00339.x.
6. Ranganathan K, Mishra G (2006) An overview of classification schemes for oral submucous fibrosis. *J. Oral Maxillofac.Pathol.* **10**: 55–58.
7. Pindborg J (1966) Oral submucous fibrosis as a precancerous condition. *J. Dent. Res.* **45**: 546–553.
8. Chen PH, Tsai CC, Lin YC, Ko YC, Yang YH, Shieh TY, Ho PS, Li CM, Min-Shan Ko A, Chen CH (2006) Ingredients contribute to variation in production of reactive oxygen species by areca quid. *J. Toxicol. Environ. Heal.* **69**: 1055–1069. https://doi.org/10.1080/15287390500360224.
9. Arakeri G, Rai KK, Hunasgi S, Merkx MAW, Gao S, Brennan PA (2017) Oral submucous fibrosis: An update on current theories of pathogenesis. *J. Oral Pathol. Med.***46**: 406–412. DOI: 10.1111/jop.12581.
10. Morgan MJ, Liu ZG (2011) Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Res.* **21**: 103–115.
11. Mithani SK, Mydlarz WK, Grumbine FL, Smith IM, Califano JA (2007) Molecular genetics of premalignant oral lesions. *Oral Dis.* **13**: 126–133. DOI: 10.1111/j.1601-0825.2006.01349.x.
12. Tsai C-H, Chou M-Y, Chang Y-C (2003) The up-regulation of cyclooxygenase-2 expression in human buccal mucosal fibroblasts by arecoline: a possible role in the pathogenesis of oral submucous fibrosis. *J. Oral Pathol. Med.* **32**: 146–153. https://doi.org/10.1034/j.1600-0714.2003.00004.x.
13. Jeng JH, Ho YS, Chan CP, Wang YJ, Hahn LJ, Lei D, Hsu CC, Chang MC (2000) Areca nut extract up-regulates prostaglandin production, cyclooxygenase-2 mRNA and protein expression of human oral keratinocytes. *Carcinogenesis* **21**: 1365–1370. https://doi.org/10.1093/carcin/21.5.365.
14. Rai V, Bose S, Saha S, Chakraborty C (2019) Evaluation of oxidative stress and the microenvironment in oral submucous fibrosis. *Heliyon* **5**: e01502. https://doi.org/10.1016/j.heliyon.2019.e01502.
15. Gupta S, Reddyand MVR, Harinath BC (2004) Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. *Indian J. Clin. Biochem.* **19**: 138–141. https://doi.org/10.1007/BF02872409.
16. Lai K-C, Lee T-C (2006) Genetic damage in cultured human keratinocytes stressed by long-term exposure to areca nut extracts. *Mutat. Res. Mol. Mech. Mutagen* **599**: 66–75. https://doi.org/10.1016/j.mrfmmm.2006.01.005.
17. Zhou ZS, Li M, Gao F, Peng JY, Xiao HB, Dai LX, Lin SR, Zhang R, Jin LY (2013) Arecoline suppresses HaCaT cell proliferation through cell cycle regulatory molecules. *Oncol. Rep.* **29**: 2438–2444. https://doi.org/10.3892/or.2013.2360.
18. Narayanan B, Narasimhan M (2015) Langerhans cell expression in oral submucous fibrosis: an immunohistochemical analysis. *J. Clin. Diagnostic Res.* **9**: ZC39.
19. Pujari R, Vidya N (2013) Mast cell density in oral submucous fibrosis : a possible role in

pathogenesis. *Int. J. Health Sci.* (Qassim) **7**: 23–29. https://doi.org/10.12816/0006017.
20. Kale AD, Mane DR, Shukla D (2013) Expression of transforming growth factor β and its correlation with lipodystrophy in oral submucous fibrosis: An immunohistochemical study. *Med. Oral Patol. Oral Cir. Bucal* **18**: e12-e18. DOI: 10.4317/medoral.18226.
21. Pant I, Rao SG, Kondaiah P (2016) Role of areca nut induced JNK/ATF2/Jun axis in the activation of TGF-β pathway in precancerous Oral Submucous Fibrosis. *Sci. Rep.* **6**: 1–15. https://doi.org/10.1038/srep34314.
22. Thomas GJ, Nystrom ML, Marshall JF (2006) αvβ6 integrin in wound healing and cancer of the oral cavity. *J. Oral Pathol. Med.* **35**: 1–10. https://doi.org/10.1111/j.1600-0714.2005.00374.x.
23. Khan I, Kumar N, Pant I, Narra S, Kondaiah P. (2012) Activation of TGF-β pathway by areca nut constituents: a possible cause of oral submucous fibrosis. *PLoS One* **7**: e51806. https://doi.org/10.1371/journal.pone.0051806.
24. Bishen KA, Radhakrishnan R, Satyamoorthy K (2008) The role of basic fibroblast growth factor in oral submucous fibrosis pathogenesis. *J. Oral Pathol. Med.* **37**: 402–411. https://doi.org/10.1111/j.1600-0714.2008.00649.x.
25. Tsai CH, Yang SF, Chen YJ, Chou MY, Chang YC (2005) The upregulation of insulin-like growth factor-1 in oral submucous fibrosis. *Oral Oncol.* **41**: 940–946. https://doi.org/10.1016/j.oraloncology.2005.05.006.
26. Deng YT, Chen HM, Cheng SJ, Chiang CP, Kuo MY (2009) Arecoline-stimulated connective tissue growth factor production in human buccal mucosal fibroblasts: Modulation by curcumin. *Oral Oncol.* **45**: e99–e105. https://doi.org/10.1016/j.oraloncology.2009.04.004.
27. Illeperuma RP, Ryu MH, Kim KY, Tilakaratne WM, Kim J (2010) Relationship of fibrosis and the expression of TGF-β1, MMP-1, and TIMP-1 with epithelial dysplasia in oral submucous fibrosis. *Oral Med. Pathol.* **15**: 21–28. https://doi.org/10.3353/omp.15.21.
28. Yang S-F, Tsai C-H, Chang Y-C (2008) The upregulation of heat shock protein 47 expression in human buccal fibroblasts stimulated with arecoline. *J. Oral Pathol. Med.* **37**: 206–210. https://doi.org/10.1111/j.1600-0714.2007.00633.x.
29. Shetty SR, Babu SG, Kumari S, Rao V, Vijay R, Karikal A (2012) Malondialdehyde levels in oral sub mucous fibrosis: A clinicopathological and biochemical study. *N. Am. J. Med. Sci.* **4**: 125–128. https://doi.org/10.4103/1947-2714.93887.
30. Ayinampudi BK, Narsimhan M (2012) Salivary copper and zinc levels in oral pre-malignant and malignant lesions. *J. Oral Maxillofac. Pathol.* **16**: 178–182. https://doi.org/10.4103/0973-029X.98452.
31. Balakrishnan C, Aswath N (2015) Estimation of serum, salivary immunoglobulin G, immunoglobulin A levels and total protein, hemoglobin in smokeless tobacco chewers and oral submucous fibrosis patients. *Contemp. Clin. Dent.* **6**: S157–S162. https://doi.org/10.4103/0976-237X.166820.
32. Santos CF, Morandini AC, Dionísio TJ, Faria FA, Lima MC, Figueiredo CM, Colombini-Ishikiriama BL, Sipert CR, Maciel RP, Akashi AP, Souza GP, Garlet GP, Rodini CO, Amaral SL, Becari C, Salgado MC, Oliveira EB, Matus I, Didier DN, Greene AS (2015) Functional local renin-angiotensin system in human and rat periodontal tissue. *PLoS One* **10**: e0134601. https://doi.org/10.1371/journal.pone.0134601.
33. Ohuchi N, Koike K, Sano M, Kusama T, Kizawa Y, Hayashi K, Taniguchi Y, Ohsawa M, Iwamoto K, Murakami H (2002) Proliferative effects of angiotensin II and endothelin-1 on

guinea pig gingival fibroblast cells in culture. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* **132**: 451–460. https://doi.org/10.1016/S1532-0456(02)00098-4.
34. Miyazaki M, Takai S, Jin D, Muramatsu M (2006) Pathological roles of angiotensin II produced by mast cell chymase and the effects of chymase inhibition in animal models. *Pharmacol. Ther.* **112**: 668–676.
35. Shi-wen X, Kennedy L, Renzoni EA, Bou-Gharios G, du Bois RM, Black CM, Denton CP, Abraham DJ, Leask A (2007) Endothelin is a downstream mediator of profibrotic responses to transforming growth factor β in human lung fibroblasts. *Arthritis Rheum.* **56**: 4189–4194. https://doi.org/10.1002/art.23134.
36. Ankita K, Shwetha V, Vanitha S, Reddy Sujatha S, Nagaraju R, Tupakula Pavan K (2019) Assessment of salivary endothelin-1 in patients with leukoplakia, submucous fibrosis, oral cancer and healthy individuals – a comparative study. *J. Stomatol. Oral. Maxillofac. Surg.* **120**: 326–331. https://doi.org/10.1016/j.jormas.2019.02.024.
37. Yanjia H, Xinchun J (2007) The role of epithelial-mesenchymal transition in oral squamous cell carcinoma and oral submucous fibrosis. *Clin. Chim. Acta.* **383**: 51–56.
38. Angadi P V, Kale AD, Hallikerimath S (2011) Evaluation of myofibroblasts in oral submucous fibrosis: Correlation with disease severity. *J. Oral Pathol. Med.* **40**: 208–213. https://doi.org/10.1111/j.1600-0714.2010.00995.x.
39. Rao AR, Das P (1989) Evaluation of the carcinogenicity of different preparations of areca nut in mice. *Int. J. Cancer* **43**: 728–732. https://doi.org/10.1002/ijc.2910430431.
40. Keshav R, Narayanappa U (2015) Expression of proliferating cell nuclear antigen (pcna) in oral submucous fibrosis: an immunohistochemical study. *J. Clin. Diagnostic Res.* **9**: ZC20.
41. Iqbal A, Tamgadge S, Tamgadge A, Pereira T, Kumar S, Acharya S, Jadhav A (2020) Evaluation of Ki-67 expression in oral submucous fibrosis and its correlation with clinical and histopathological features. *J. Microsc. Ultrastruct.* **8**: 20. https://doi.org/10.4103/jmau.jmau_28_19.
42. Pereira T, Surve R, Shetty S, Gotmare S (2020) Qualitative expression of hypoxia-inducible factor-1α in malignant transformation of oral submucous fibrosis: An immunohistochemical study. *J. Oral Maxillofac. Pathol.* **24**: 106. https://doi.org/10.4103/jomfp.JOMFP_234_19.
43. Jirge V, Shashikanth M, Ali I, Anshumalee N (2008) Levamisole and antioxidants in the management of oral submucous fibrosis: a comparative study. *J. Indian Acad. Oral Med. Radiol.* **20**: 135. https://doi.org/10.4103/0972-1363.52827.
44. Shetty P, Shenai P, Chatra L, Rao PK (2013) Efficacy of spirulina as an antioxidant adjuvant to corticosteroid injection in management of oral submucous fibrosis. *Indian J. Dent. Res.* **24**: 347–350. https://doi.org/10.4103/0970-9290.118001.
45. Singh M, Niranjan HS, Mehrotra R, Sharma D, Gupta SC (2010) Efficacy of hydrocortisone acetate/hyaluronidase vs triamcinolone acetonide/hyaluronidase in the treatment of oral submucous fibrosis. *Indian J. Med. Res.***131**: 665–669.
46. Alshadwi A, Bhatia I (2012) Excision of oral submucous fibrosis and reconstruction with full thickness skin graft: a case study and review of the literature. *Case Rep. Dent.* **2012**: 628301.https://doi.org/10.1155/2012/628301.
47. Gupta A, Kukkar N, Sharif K, Main BJ, Albers CE, El-Amin III SF (2015) Bone graft substitutes for spine fusion: A brief review. *World J. Orthop.* **6**: 449–456. https://doi.org/10.5312/wjo.v6.i6.449.
48. Arakeri G, Patil S, Maddur N, Rao US V, Subash A, Patil S, Gao S, Brennan PA (2020) Long-term effectiveness of lycopene in the management of oral submucous fibrosis

(OSMF): A 3-years follow-up study. *J. Oral Pathol. Med.* **49**: 803–808. https://doi.org/10.1111/jop.13085.
49. Zechmeister L (1944) Cis-trans isomerization and stereochemistry of carotenoids and diphenyl-polyenes. *Chem. Rev.* **34**: 267–344. https://doi.org/10.1021/cr60108a004.
50. Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* **274**: 532–538. https://doi.org/10.1016/0003-9861(89)90467-0.
51. Pennathur S, Maitra D, Byun J, Sliskovic I, Abdulhamid I, Saed GM, Diamond MP, Abu-Soud HM (2010) Potent antioxidative activity of lycopene: A potential role in scavenging hypochlorous acid. *Free Radic. Biol. Med.* **49**: 205–213. https://doi.org/10.1016/j.freeradbiomed.2010.04.003.
52. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L (2016) Melatonin as an antioxidant: under promises but over delivers. *J. Pineal Res.* **61**: 253–278.
53. Giannoulia-Karantana A, Vlachou A, Polychronopoulou S, Papassotiriou I, Chrousos GP (2007) Melatonin and immunomodulation: connections and potential clinical applications. *Neuroimmunomodulation* **13**:133–144.
54. Reiter RJ (1991) Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr. Rev.* **12**:151–180. https://doi.org/10.1210/edrv-12-2-151.
55. Sugden D (1989) Melatonin biosynthesis in the mammalian pineal gland. *Experientia* **45**: 922–932.
56. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ (2007) One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.* **42**: 28–42.
57. Shimozuma M, Tokuyama R, Tatehara S, Umeki H, Ide S, Mishima K, Saito I, Satomura K (2011) Expression and cellular localizaion of melatonin-synthesizing enzymes in rat and human salivary glands. *Histochem. Cell Biol.* **135**: 389–396. https://doi.org/10.1007/s00418-011-0800-8.
58. Madapusi BT, Rao SR (2018) Preliminary evaluation of human gingiva as an extrapineal site of melatonin biosynthesis in states of periodontal health and disease. *J. Clin. Diagnostic Res.***12**: ZF01–ZF07. https://doi.org/10.7860/JCDR/2018/32451.11078.
59. Cagnacci A (1997) Influences of melatonin on human circadian rhythms. *Chronobiol. Int.* **14**: 205–220.
60. Sharma S, Haldar C (2006) Melatonin prevents X-ray irradiation induced oxidative damagein peripheral blood and spleen of the seasonally breeding rodent, Funambulus pennanti during reproductively active phase. *Int. J. Radiat. Biol.* **82**: 411–419. https://doi.org/10.1080/09553000600774105.
61. Tan D-X, Manchester LC, Reiter RJ, Plummer BF, Hardies LJ, Weintraub ST, Shepherd AM (1998) A novel melatonin metabolite, cyclic 3-hydroxymelatonin: abiomarker ofin vivohydroxyl radical generation. *Biochem. Biophys. Res. Commun.* **253**: 614–620. https://doi.org/10.1006/bbrc.1998.9826.
62. Ressmeyer A-R, Mayo JC, Zelosko V, Sáinz RM, Tan DX, Poeggeler B, Antolín I, Zsizsik BK, Reiter RJ, Hardeland R (2003) Antioxidant properties of the melatonin metabolite N 1 -acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox. Rep.* **8**: 205–213. https://doi.org/10.1179/135100003225002709.
63. Xia M-Z, Liang Y-L, Wang H, Chen X, Huang YY, Zhang ZH, Chen YH, Zhang C, Zhao M, Xu DX, Song LH (2012) Melatonin modulates TLR4-mediated inflammatory genes

through MyD88- and TRIF-dependent signaling pathways in lipopolysaccharide-stimulated RAW264.7 cells. *J. Pineal Res.* **53**: 325–334. https://doi.org/10.1111/j.1600-079X.2012.01002.x.

64. Deng W-G, Tang S-T, Tseng H-P, Wu KK (2006) Melatonin suppresses macrophage cyclooxygenase-2 and inducible nitric oxide synthase expression by inhibiting p52 acetylation and binding. *Blood* **108**: 518–524. https://doi.org/10.1182/blood-2005-09-3691.
65. Zhao X, Sun J, Su W, Shan H, Zhang B, Wang Y, Shabanova A, Shan H, Liang H (2018) Melatonin protects against lung fibrosis by regulating the hippo/YAP pathway. *Int. J. Mol. Sci.* **19**: 1118. https://doi.org/10.3390/ijms19041118.
66. Lebda MA, Sadek KM, Abouzed TK, Tohamy HG, El-Sayed YS (2018) Melatonin mitigates thioacetamide-induced hepatic fibrosis via antioxidant activity and modulation of proinflammatory cytokines and fibrogenic genes. *Life Sci.* **192**: 136–143. https://doi.org/10.1016/j.lfs.2017.11.036.
67. Wang H, Wei W, Wang NP, Gui SY, Wu L, Sun WY, Xu SY (2005) Melatonin ameliorates carbon tetrachloride-induced hepatic fibrogenesis in rats via inhibition of oxidative stress. *Life Sci.* **77**: 1902–1915. https://doi.org/10.1016/j.lfs.2005.04.013.
68. Tahan V, Ozaras R, Canbakan B, Uzun H, Aydin S, Yildirim B, Aytekin H, Ozbay G, Mert A, Senturk H (2004) Melatonin reduces dimethylnitrosamine-induced liver fibrosis in rats. *J. Pineal Res.* **37**: 78–84. https://doi.org/10.1111/j.1600-079X.2004.00137.x.
69. Czechowska G, Celinski K, Korolczuk A, Wojcicka G, Dudka J, Bojarska A, Reiter RJ (2015) Protective effects of melatonin against thioacetamide-induced liver fibrosis in rats. *J. Physiol. Pharmacol.* **66**: 567–579.
70. Lee EJ, Lee MY, Chen HY, Hsu YS, Wu TS, Chen ST, Chang GL (2005) Melatonin attenuates gray and white matter damage in a mouse model of transient focal cerebral ischemia. *J. Pineal Res.* **38**: 42–52. https://doi.org/10.1111/j.1600-079X.2004.00173.x.
71. Çikler E, Ercan F, Çetinel Ş, Contuk G, Şener G (2005) The protective effects of melatonin against water avoidance stress-induced mast cell degranulation in dermis. *Acta Histochem.* **106**: 467–475. https://doi.org/10.1016/j.acthis.2004.10.001.
72. Prendergast BJ, Cable EJ, Patel PN, Pyter LM, Onishi KG, Stevenson TJ, Ruby NF, Bradley SP (2013) Impaired leukocyte trafficking and skin inflammatory responses in hamsters lacking a functional circadian system. *Brain Behav. Immun.* **32**: 94–104. https://doi.org/10.1016/j.bbi.2013.02.007.
73. Wang Y rong, Hong R tao, Xie Y yuan, Xu J ming (2018) Melatonin ameliorates liver fibrosis induced by carbon tetrachloride in rats via inhibiting TGF-β1/Smad signaling pathway. *Curr. Med. Sci.* **38**: 236–244. https://doi.org/10.1007/s11596-018-1871-8.
74. Osbek E, Ilbey YO, Ozbek M, Simsek A, Cekmen M, Somay A (2009) melatonin attenuates unilateral ureteral obstruction–induced renal injury by reducing. *J. Endourol.* **23**: 1165–1173.
75. Choi HS, Kang JW, Lee SM (2015) Melatonin attenuates carbon tetrachloride-induced liver fibrosis via inhibition of necroptosis. *Transl. Res.* **166**: 292–303. https://doi.org/10.1016/j.trsl.2015.04.002.
76. Turgut M, Öktem G, Uysal A, Yurtseven ME (2006) Immunohistochemical profile of transforming growth factor-β1 and basic fibroblast growth factor in sciatic nerve anastomosis following pinealectomy and exogenous melatonin administration in rats. *J. Clin. Neurosci.* **13**: 753–758. https://doi.org/10.1016/j.jocn.2005.07.019.
77. Crespo I, San-Miguel B, Fernández A, De Urbina JO, González-Gallego J, Tuñón MJ (2015)

Melatonin limits the expression of profibrogenic genes and ameliorates the progression of hepatic fibrosis in mice. *Transl. Res.* **165**: 346–357. https://doi.org/10.1016/j.trsl.2014.10.003.

78. Hosseinzadeh A, Javad-Moosavi SA, Reiter RJ, Yarahmadi R, Ghaznavi H, Mehrzadi S (2018) Oxidative/nitrosative stress, autophagy and apoptosis as therapeutic targets of melatonin in idiopathic pulmonary fibrosis. *Expert Opin. Ther. Targets* **22**:1049–1061.
79. Parmar P, Limson J, Nyokong T, Daya S (2002) Melatonin protects against copper-mediated free radical damage. *J. Pineal Res.* **32**: 237–242. https://doi.org/10.1034/j.1600-079X.2002.01859.x.
80. Sharma R, Reiter RJ, Ma Q (2019) Melatonin: a hypothesis regarding its use to treat Wilson disease. *Med. Hypotheses* **133**: 109408. https://doi.org/10.1016/j.mehy.2019.109408.
81. Lin GJ, Huang SH, Chen SJ, Wang CH, Chang DM, Sytwu HK (2013) Modulation by melatonin of the pathogenesis of inflammatory autoimmune diseases. *Int. J. Mol. Sci.* **14**:11742–11766.
82. Ohashi N, Ishigaki S, Isobe S (2019) The pivotal role of melatonin in ameliorating chronic kidney disease by suppression of the renin–angiotensin system in the kidney. *Hypertens. Res.* **42**:761–768.
83. León J, Casado J, Jiménez Ruiz SM, Zurita MS, González-Puga C, Rejón JD, Gila A, Muñoz de Rueda P, Pavón EJ, Reiter RJ, Ruiz-Extremera A (2014) Melatonin reduces endothelin-1 expression and secretion in colon cancer cells through the inactivation of FoxO-1 and NF-κβ. *J. Pineal Res.* **56**: 415–426. https://doi.org/10.1111/jpi.12131.
84. Yu N, Sun YT, Su XM, He M, Dai B, Kang J (2016) Melatonin attenuates TGFβ1-induced epithelial-mesenchymal transition in lung alveolar epithelial cells. *Mol. Med. Rep.* **14**: 5567–5572. https://doi.org/10.3892/mmr.2016.5950.
85. Jung-Hynes B, Schmit TL, Reagan-Shaw SR, Siddiqui IA, Mukhtar H, Ahmad N (2011) Melatonin, a novel Sirt1 inhibitor, imparts antiproliferative effects against prostate cancer in vitro in culture and in vivo in TRAMP model. *J. Pineal Res.* **50**: 140–149. https://doi.org/10.1111/j.1600-079X.2010.00823.x.
86. Park SY, Jang WJ, Yi EY, Jang JY, Jung Y, Jeong JW, Kim YJ (2010) Melatonin suppresses tumor angiogenesis by inhibiting HIF-1α stabilization under hypoxia. *J. Pineal Res.* **48**: 178–184. https://doi.org/10.1111/j.1600-079X.2009.00742.x.

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