

Review

Etiology of Oral Potentially Malignant Disorders and Squamous Cell Carcinoma Based on Cellular Stress Regulation and Matrix Stiffness

Toshihiro Hasegawa^{1,2,†}, Takehito Ouchi^{1,3,*}, Yoshiyuki Shibukawa³, Seiji Asoda¹,
Taneaki Nakagawa¹¹Department of Dentistry and Oral Surgery, Keio University School of Medicine, 160-8582 Tokyo, Japan²Department of Oral and Maxillofacial Surgery, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, 113-0021 Tokyo, Japan³Department of Physiology, Tokyo Dental College, 101-0061 Tokyo, Japan*Correspondence: takehito@tdc.ac.jp (Takehito Ouchi)

†These authors contributed equally.

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Abstract

The oral cavity serves as the initial segment of the digestive system and is responsible for both nutritional supplementation and the mechanical breakdown of food. It comprises distinct hard and soft tissues; the oral mucosa is subject to mechanical stress and interaction with microbiota. In oral cancer, tumors exhibit abnormal cellular networks and aberrant cell-cell interactions arising from complex interplays between environmental and genetic factors. This presents a challenge for clinicians and researchers, impeding the understanding of mechanisms driving oral cancer development and treatment strategies. Lesions with dysplastic features are categorized under oral potentially malignant disorders, including oral leukoplakia, erythroplakia, oral submucous fibrosis, and proliferative verrucous leukoplakia, carrying a high malignancy risk. In this review, we discuss oral cancer cell characteristics and the stiffness of the surrounding matrix. We also discuss the significance of stiffness equilibrium in oral potentially malignant disorders, particularly oral submucous fibrosis, possibly triggered by mechanical stress such as betel quid chewing.

Keywords: oral cancer; matrix stiffness; extracellular matrix; metastasis

1. Introduction

Cancer cells manifest six distinct biological characteristics that are acquired throughout the multistage development of human tumors. These include (1) the sustenance of growth signaling, (2) the evasion of growth inhibitors, (3) cell death resistance, (4) replication immortality, (5) initiation of angiogenesis, and (6) the activation of infiltration and metastasis. Recent conceptual progress has supplemented this list with two additional traits: energy metabolism reprogramming and evasion of immune destruction. These features serve as foundational principles that streamline the complex nature of neoplastic diseases. These features are characterized by genomic instability, generating genetic diversity, which consequently facilitates their acquisition, as well as inflammation that contributes to diverse characteristics and functions [1]. In this context, various stresses, such as oxidative stress [2] and matrix stiffness [3], inevitably connect them, thereby complicating the determination of clinical cancer progression and outcome. Mechanical signaling triggers mitochondrial and metabolic reprogramming, enabling cells to adapt to oxidative stress [4]. Matrix stiffness is a critical factor in cancer progression and is caused by the accumulation, contraction, and cross-linking of the extracellular matrix (ECM) by cancer and stromal cells. These cells respond

to matrix stiffness, which determines their phenotype. Furthermore, matrix stiffness activates and/or deactivates specific transcription factors within cancer and stromal cells, consequently regulating cancer progression [5].

Oral submucous fibrosis (OSMF) is an oral potentially malignant disorders (OPMDs) [6]. Betel quid (BQ) chewing serves as a potential risk factor, exacerbating the propensity for oral cancer and OSMF, a premalignant oral condition with malignant transformation potential. The constituents of BQ, such as areca nuts, coarse areca nut fiber-induced trauma, catechin, copper, alkaloids, reactive oxygen species (ROS) induction, inflammation, and cytotoxicity, are likely contributory factors [7,8]. These components potentially incite tissue inflammation, stimulate fibroblast proliferation, induce collagen deposition, trigger myofibroblast differentiation and contraction, promote collagen cross-linking, and hinder collagen phagocytosis, ultimately culminating in OSMF and oral cancer. These processes are orchestrated by changes induced by BQ components in collagen-related factors, such as lysyl oxidase (LO) [9]. Moreover, BQ components exhibit genotoxicity, potentially altering DNA, protein, and lipid structures, resulting in antigen production. Additionally, BQ components induce keratinocyte inflammation by stimulating the production of prostaglandins, TNF- α , IL-6, IL-



8, and granulocyte-macrophage colony-stimulating factors in keratinocytes [10]. The risk of malignant transformation among OPMD subtypes varies, with leukoplakia having the lowest risk, followed by intermediate-risk groups comprising OSMF, erythroleukoplakia, and erythroplakia, and exophytic verrucous hyperplasia carrying the highest risk. Alcohol consumption significantly promotes OSMF, whereas BQ chewing accelerates the malignant transformation of exophytic verrucous hyperplasia. These insights can help prevent the occurrence of oral cancer at both individual and population levels [7]. Thus, several factors can induce intracellular and extracellular plasticity, leading to the emergence of OSMF and cancer formation. Anura *et al.* [11] employed atomic force microscopy (AFM) to explore tissue topography at micro/nano levels. Their observations revealed a densely packed parallel alignment of collagen fibers in OSMF tissue compared to normal tissue. AFM-based indentation revealed diminished flexibility in the subepithelium of OSMF tissue, characterized by increased Young's modulus, stiffness, and adhesiveness, coupled with reduced deformation of the juxta-epithelial connective tissue towards deeper layers. These nanomechanical variances bear potential implications for the pathophysiological microenvironment. Excessive collagen I deposition, reduced expression of collagen III and fibronectin, and the presence of α -SMA positive myofibroblasts in OSMF underscore its pathological basis and highlight the impact of altered ECM. The mechanobiological shifts in OSMF align with changes in collagen composition identified using immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR). The comparative nanomechanical profiles of normal oral mucosa and OSMF, juxtaposed with their structural and cardinal molecular attributes, serve as a pivotal tool in developing comprehensive pathobiological insights into the conceivable links underlying the malignant transformation of this pre-cancerous condition [11].

2. Mechanical Regulation

2.1 Matrix and Cellular Mechanics

Tumor cells encounter diverse mechanical stimuli within the tumor microenvironment, including cell-cell and cell-ECM tension, compression stress from expanding tumor masses, interstitial fluid pressure, and shear stress. These stimuli exhibit a positive feedback relationship with cellular responses, such as heightened actomyosin contractility and stiffening of the extracellular matrix, which exacerbate tumor progression and aggressiveness. A comprehensive understanding of the interplay between the components of the tumor microenvironment and the molecular mechanisms governing cellular reactions to mechanical inputs is pivotal for developing efficacious cancer treatments [12].

Metastasis is a pivotal aspect of cancer progression. Tumor cells metastasize from the primary tumor to distant organs, resulting in a life-threatening condition. Distinct

biomechanical properties are responsible for tumor cell growth, invasion, intravascular invasion, circulation, arrest/adhesion, and extravascular invasion, collectively constituting the metastatic cascade. The basement membrane (BM) is a specialized extracellular matrix that serves as a major barrier that cancer cells must repeatedly overcome to metastasize [13].

Fiore *et al.* [14] proposed that mechanical forces exerted both above and below the progenitor cells in multi-layer epithelia augment the formation of precancerous tumor structures that significantly impact tumor progression. Cellular transformation accompanies molecular changes altering cytoskeletal organization; therefore, cell stiffness must be evaluated to potentially facilitate the detection and assessment of cancer cells [15]. The ECM is the critical component of tumors, serving diverse roles, including mechanical support, microenvironmental modulation, and generation of signaling molecules. The quantity and cross-linking status of ECM components primarily determine tissue stiffness. During tumorigenesis, interactions between cancer cells and the tumor microenvironment (TME) frequently result in ECM rigidity, inducing aberrant mechanotransduction and further malignant transformations. A comprehensive understanding of ECM dysregulation within the TME is instrumental in identifying promising targets for cancer therapy [16]. Thus, stiffness exerts its influence on cellular components and the ECM in various regional cancers, including oral cancers [17,18].

Metastatic cells typically exhibit lower stiffness than non-malignant tumor cells, with the high deformability of both the cell and its nucleus presumed to confer a significant advantage for their metastatic potential. However, the question of whether there exists a finely calibrated yet fixed mechanical state accommodating all requisite mechanical attributes for metastatic cell survival across the cascade or whether tumor cells dynamically refine their properties and intracellular components upon encountering each new stage remains unresolved [19]. Engler *et al.* [20] have demonstrated the impact of matrix elasticity on human mesenchymal stem cell (MSC) differentiation, yielding valuable insights into the physical effects of the *in vivo* microenvironment and the therapeutic potential of MSCs. Stiffness regulation extends beyond MSCs to encompass various cell types. In the vascular microenvironment, stiffness and fluid shear stress engage in a signaling interplay, with matrices mirroring the properties of young, healthy vessels effectively inducing fluid shear stress-mediated atheroprotection. These findings imply that targeting intimal stiffening and/or the endothelial cell response to intimal stiffening might enhance vascular health [21]. As tumor progression advances, the composition and physical attributes of the ECM undergo alteration. Elevated matrix stiffness notably influences tumor growth and metastasis. In particular, endothelial cells play a pivotal role in cancer progression; however, the impact of tumor stiffness on the endothe-

lium and its metastasis-related effects remain unexplored. While stiffness is a trigger for malignant transformation, other contributing factors should not be overlooked.

OPMDs frequently precede oral cancer. Wang *et al.* [22] examined the rate and duration of malignant transformation among different OPMDs in a patient cohort. Epithelial dysplastic lesions exhibited a 1.89-fold higher risk of malignant transformation compared with that of non-dysplastic lesions. The anatomical site of OPMDs and the presence of epithelial dysplasia demonstrated significant associations with malignant transformation. Tongue lesions carried a hazard rate ratio 1.87 times higher than that for buccal lesions. These findings highlight the need for prolonged clinical follow-up of patients with OPMDs, with histopathological evaluation serving as a vital predictor of cancer development, enabling the monitoring of potential malignant transformation [22].

The alteration and functional defects in lymphocyte populations have been observed in different stages of oral cancer. Yeh *et al.* [23] focused on the lymphocyte population and investigated whether CD4⁺ T-helper cells, CD8⁺ T-cells, CD19⁺ B cells, and CD56⁺ natural killer (NK) cells, along with their activation markers (CD25⁺ and CD69⁺) present at the initial diagnostic stage of oral precancer impact future malignant transformation. Additionally, the authors explored the relationship between age, oral habits, and the malignant transformation of oral precancers. Given the multifactorial etiology of oral cancer, incorporating age, alcohol consumption, and cellular immune responses into the context of their effects on malignant transformation offers insights into the pathogenesis of oral squamous cell carcinoma (OSCC). The expression of CD4⁺CD69⁺, CD19⁺CD69⁺, and CD56⁺CD69⁺ lymphocytes is pivotal in this process, underscoring the significance of cellular immunity in OSCC pathogenesis. The peripheral blood lymphocyte markers in precancer patients might serve as predictive and diagnostic tools for monitoring the potential for malignant changes in OSCC [23].

The mechanism of OSMF and its potential transformation into OSCC have been explored in previous studies. Key molecules and abnormal RNAs that have substantial implications for disease onset, progression, detection, diagnosis, monitoring, and prognosis have been identified [24]. Consequently, advanced diagnostic techniques have been employed to predict disease progression and assess the risk of malignant transformation, thereby providing insights into the importance of early detection and prevention of OPMDs [25].

Using quantitative mass spectroscopy, Reid *et al.* [26] observed that the matricellular protein CCN1/CYR61 is tightly regulated by endothelial cell stiffness. This regulation prompts stiffness-induced CCN1 to trigger β -catenin nuclear translocation and signaling, consequently elevating N-cadherin levels on the endothelial surface. This, in turn, promotes N-cadherin-dependent cancer cell–

endothelial cell interactions. CCN1 knockout in endothelial cells inhibits its binding to blood vessels, an instrumental step in the metastasis of melanoma cells through the vasculature. Targeting alterations induced by vascular stiffness, such as those involving CCN1, presents an unexplored avenue to inhibit metastasis [26]. N-cadherin and other cadherin types, including E-cadherin, serve as epithelial markers and products of the *CDH1*. E-cadherin, renowned for its role in epithelial-mesenchymal transition [27], is subject to epigenetic regulation through DNA hypermethylation, an epigenetic mechanism silencing various genes, including tumor suppressors, and is frequently implicated in human cancers [28]. Epigenetic regulation is also involved in the epithelial-mesenchymal transition (EMT)-related genes, including *CDH1* [29,30].

FOXA2 bound to the *CDH1* promoter augments the expression of E-cadherin, leading to diminished cancer cell migration. Elevated FOXA2 expression in oral cancer tissues was associated with heightened E-cadherin expression, decreased lymph node metastasis, and improved patient survival. This FOXA2–E-cadherin is involved in regulating oral cancer cell metastasis, thereby shedding light on the tumor-suppressive activity of FOXA2 in oral cancer [27]. *CDH1* mutations have been identified by Er *et al.* [31] using next-generation sequencing in 50 formalin-fixed paraffin-embedded tumor specimens obtained from patients with oral squamous cell carcinoma. The causative factors triggering these phenomena within cadherin expression patterns and mutations warrant further investigation to confirm the role of mechanical stress regulation.

2.2 Mechanosensing in Cancer

Numerous physical factors exert influence on tumor progression, including increased matrix deposition that augments tumor stiffness, matrix remodeling owing to forces from cancer cells and stromal fibroblasts, matrix cross-linking, increased cellularity, and the accumulation of both solid and stromal pressure. Mechanosensitive ion channels of the Piezo-type (PIEZO channels, including PIEZO1 and PIEZO2) have been identified as ion channels activated by mechanical stimuli [32]. The Hippo pathway, responsive to extracellular conditions, governs cell proliferation via the nuclear translocation of downstream effectors, yes-associated protein (YAP), and transcriptional coactivator with PDZ-binding motifs (TAZ), thereby inducing target gene transcription [33,34]. Hippo/YAP signaling is potentially a therapeutic target for treating squamous cell carcinoma [35]. Xu *et al.* [36] have reported that the fibrotic matrix prompts mesenchymal transformation of epithelial cells in OSMF. Elevated stiffness of the fibrotic matrix in OSMF leads to increased proliferation and epithelial-mesenchymal transition (EMT) of mucosal epithelial cells, wherein the PIEZO1 and YAP axes play vital roles in mechanical sensing and signal transduction [36]. Hiemer *et al.* [37] demonstrated that nuclear YAP and TAZ, which

are well-known mechanotransducers, stimulate OSCC cell proliferation, survival, and migration. Global gene expression profiles following YAP and TAZ knockdown demonstrated changes in the regulation of genes implicated in pro-tumorigenic signaling, including those essential for cell cycle progression and survival. Notably, the transcriptional signature governed by YAP and TAZ strongly correlated with gene expression alterations observed in human OSCC identified by The Cancer Genome Atlas (TCGA), highlighting the central roles of YAP and TAZ in OSCC biology [37]. Hasegawa *et al.* [38] examined the impact of YAP signaling on OSCC tumorigenesis. Loss-of-function experiments utilizing siRNA and inhibitors, coupled with immunohistochemical analysis of tissue specimens from patients with OSCC, revealed the involvement of YAP signaling in OSCC cell proliferation. Furthermore, they discovered that the calcium channel PIEZO1 is a transcriptional target of YAP signaling, implying that elevated PIEZO1 expression facilitates PIEZO1 agonist-dependent calcium influx and OSCC cell proliferation. Immunohistochemistry revealed YAP overexpression in the nucleus and/or cytoplasm of tumor cells. While PIEZO1 and Ki-67 were frequently expressed in OSCC specimens, their presence was negligible in non-tumor areas. These findings suggest that the YAP/PIEZO1 axis fosters OSCC cell proliferation [38]. This pathway has been linked to tumorigenesis in OSCC and hepatocellular carcinoma while also correlating with poorer prognoses in breast, ovarian, and hepatocellular carcinoma [39–41].

Transient receptor potential vanilloid 4 (TRPV4), a physicochemical stress-sensitive ion channel, plays a pivotal role in the nuclear translocation of YAP/TAZ in response to matrix stiffness and TGF β 1 in normal mouse primary epidermal keratinocytes. Deletion of TRPV4 inhibits matrix stiffness- and TGF β 1-induced activation of YAP/TAZ proteins and AKT, but not Smad2/3, indicating the regulatory role of TRPV4 in EMT in normal mouse primary epidermal keratinocytes [42,43]. Chu *et al.* [44] investigated the antitumor activity and underlying mechanisms of {[N-(4-(5-(3-(3-(4-acetamido-3-(trifluoromethyl)phenyl)ureido)phenyl)-1,2,4-oxadiazol-3-yl)-3-chlorophenyl)-nicotinamide]} (ATN), a novel YAP inhibitor, in OSCC cells and demonstrated its varying antiproliferative efficacy against OSCC cells relative to non-tumorigenic human fibroblast cells. Additionally, ATN effectively suppressed YAP expression and its downstream targets, including Akt, p-AMPK, c-Myc, and cyclin D1, mirroring the antiproliferative efficacy of ATN. Concordant to the role of YAP in regulating cancer cell survival and migration, ATN induced caspase-dependent apoptosis and inhibited migration in OSCC. Mechanistically, the antitumor activity of ATN in OSCC was partly attributed to its regulation of Mcl-1 expression. Collectively, these findings highlight the translational potential of YAP inhibitors, represented by ATN, as viable anticancer therapies

for OSCC [44]. Thus, mechanosensitive ion channels and mechanotransducers hold promise as contributors to cancer development and metastasis.

2.3 Mechanical Stress and Oxidative Stress

Matrix stiffness plays a crucial role in oral tissues and other ECM stiffness, a crucial mechanical attribute of the nucleus pulposus (NP) tissue, contributing to intervertebral disc degeneration (IDD) pathogenesis. Wang *et al.* [45] reported elevated PIEZO1 expression and increased ECM elasticity modulus in degenerative NP tissues. Stiff ECM activates the PIEZO1 channel, leading to elevated intracellular Ca²⁺ levels. Additionally, PIEZO1 activation enhances intracellular ROS levels and the expression of GRP78 and CHOP, contributing to oxidative and endoplasmic reticulum (ER) stresses. Furthermore, a stiff ECM exacerbates oxidative stress-induced senescence and apoptosis in human NP cells. Inhibiting PIEZO1 can potentially mitigate oxidative stress-induced senescence and apoptosis stemming from increased ECM stiffness [45]. Alterations in the physical properties of the extracellular matrix are observed in cancerous and aging tissues. Tharp *et al.* [4] observed that mechanosignaling through adhesion influences cellular metabolic programs. Mitochondrial stress responses, mediated by mechanosensitive ion exchange and heat shock factor 1, modulate the metabolic programs of cells adapting to diverse mechanical microenvironments. These findings substantiate the metabolic phenotypes evident in some epithelial cells within fibrotic/sclerotic tumor tissue microenvironments [4].

2.4 Mechanical Regulation in Cancer

The physical characteristics of a tumor are intricately linked to its phenotype and pose challenges during treatment. Increased stiffness applies forces on cancer cells, intensifying tumor invasiveness and compromising therapeutic efficacy [46]. Although solid tumor metastasis arises from various genetic programs, the result is consistent. Tumor cells evade the primary tumor and disseminate to distant organs by overcoming a series of physical barriers [47]. Consequently, heightened physical pressure can augment stem cell tumor properties. Genetic regulation offers an effective approach to controlling stiffness. Genetic alterations in basement membrane stiffness increase membrane tension computationally and accelerate invasive squamous cell carcinoma progression *in vivo*. Mechanical forces exerted above and below the progenitors of multilayered epithelia may reportedly shape premalignant tumor architecture and influence tumor progression [14]. As cancer develops in the epithelium and often infiltrates the mesenchymal connective tissues, cancer-associated fibroblasts within the cancerous mesenchyme exhibit characteristics reminiscent of mesenchymal stem cells and play a pivotal role in the malignant transformation of oral mucosa through the regulation of T-cell proliferation [48].

Only a minority of patients with cancer robustly respond to immune checkpoint inhibitors. This limited success may partly be attributed to the dense ECM that forms a barrier for T-cells. By comparing five preclinical mouse tumor models with heterogeneous tumor microenvironments, Nicolas-Boluda *et al.* [49] investigated the rate of tumor stiffening, ECM structure remodeling, and the impact of these factors on intratumoral T-cell migration. A strategy targeting ECM through LO inhibition indicated that *in vivo* stiffness correlated positively with tumor growth and ECM cross-linking but negatively with T-cell migration. Furthermore, inhibiting collagen stabilization reduced ECM volume and tumor stiffness while boosting T-cell migration and enhancing the efficacy of anti-PD-1 blockade. These findings provide mechanistic insights into the characteristics of solid tumors and aid in understanding immunotherapy resistance and a rationale for integrating ECM-targeted therapeutic approaches with anti-PD-1 therapy [49].

LO, a copper-dependent amine oxidase, substantially contributes to the biogenesis of connective tissue matrices by cross-linking ECM proteins such as collagen and elastin. LO levels increase in numerous fibrotic conditions [50]. LO catalyzes the oxidative deamination of lysine residues in elastin and collagens, initiating their assembly into insoluble fibers within the extracellular matrix [51]. Trivedy *et al.* [52] reported upregulated LO expression in OSMF and OSCC. By examining oral biopsies from patients with OSMF, OSCC arising in OSMF, and OSCC unrelated to OSMF, LO co-localized with strongly stained collagen and elastin areas in histochemical stainings. SCC tissues revealed LO localization adjacent to invading epithelial islands, indicating stromal reactions in both OSMF-related and non-OSMF-related SCC cases. These findings suggest that LO upregulation may be vital in OSMF pathogenesis and early stromal reactions in oral cancer [52]. The high copper content in areca ingredients has been demonstrated [53,54]. Elevated soluble copper levels found in oral fluids of chronic chewers further support the role of copper as an initiating factor in OSMF, stimulating fibrosis by upregulating LO activity [52–54]. Areca and copper can induce LO, and LO possesses transcription activator functions that can activate collagen III and elastin promoters in OSMF [52]. The substantial copper release from areca products is believed to induce LO activity, enhancing collagen synthesis by fibroblasts, promoting cross-linking, and inhibiting degradation [55,56].

Cancer cells experience mechanical stress within their primary environments as well as during their migration through the bloodstream and various intravascular stages of the metastatic cascade [57]. As tumor cells circulate in the bloodstream or lymphatic vessels, they endure diverse mechanical stresses due to hemodynamic forces and vasoconstriction. Circulating tumor cells (CTCs) must withstand these stresses for the metastatic cascade to be successful (Fig. 1). High fluid shear stress levels can induce cell cycle

arrest [58] and even lead to the destruction of circulating tumor cells [59]. Consequently, only a small fraction of CTCs can evade the effects of fluid shear stress and adhere to the endothelium. CTC clusters have been observed traversing capillary-sized vessels [60].

Tumor stiffness is associated with increased interstitial tissue pressure and solid stress stemming from disrupted vasculature, tumor expansion [61], and fibrosis [62]. Solid cancer progression frequently accompanies an increase in matrix stiffness, primarily owing to the deposition of large amounts of ECM proteins such as collagen, fibronectin, or hyaluronic acid by activated stromal fibroblasts [63]. Paszek *et al.* [64] noted that matrix stiffness triggers intracellular signaling and malignant transformation. Briefly, matrix stiffness can potentiate tumor malignancy. Additionally, tumor stiffness can impact treatment effectiveness. Solid tumors often exhibit intrinsic resistance to treatment, partly attributed to the hindrance of blood-borne drug delivery to cancer cells [65,66]. Tumor and stromal cells generate a matrix comprising collagens, proteoglycans, and other molecules that impede macromolecule transport. This matrix stiffness contributes to resistance. Netti *et al.* [67] demonstrated that collagen organization and the combination of collagen and proteoglycans significantly contribute to interstitial transport resistance in human colon adenocarcinoma, human glioblastoma, human soft tissue sarcoma, and murine mammary carcinoma. When hepatocellular carcinoma (HCC) cells were cultured in media with varying matrix stiffness, cells cultured in the stiffest medium exhibited the highest resistance to paclitaxel, 5-FU, and cisplatin. These findings indicate that matrix stiffness substantially influences the chemoresistance of HCC cells [68]. We propose that matrix stiffness constitutes an aspect of chemoresistance. Consequently, novel anticancer therapies, such as gene therapy and immunotherapy, might be ineffective owing to their status as high-molecular-weight drugs that cannot effectively permeate the tumor stroma.

3. Discussion

Oral cancer, which originates from the mucosal epithelium of the oral cavity, represents the most prevalent form of head and neck cancer. The incidence of oral cancer varies across countries or regions and is typically associated with exposure to tobacco-derived carcinogens, excessive alcohol consumption, or a combination of both [69]. Notably, the *de novo* type characterized by induration highlights the impact of tumor invasion depth on metastasis and prognosis [70]. To advance oral cancer treatment, comprehending the complex interplay between genetic regulation, tissue stiffness, and environmental factors within the host environment is crucial. Functional analysis and tumor structure evaluation serve as vital tools in cancer treatment. The breakdown of the basement membrane, resulting from tissue force and protease enzyme digestion of the ECM, is responsible for the invasion of the mesenchyme and the

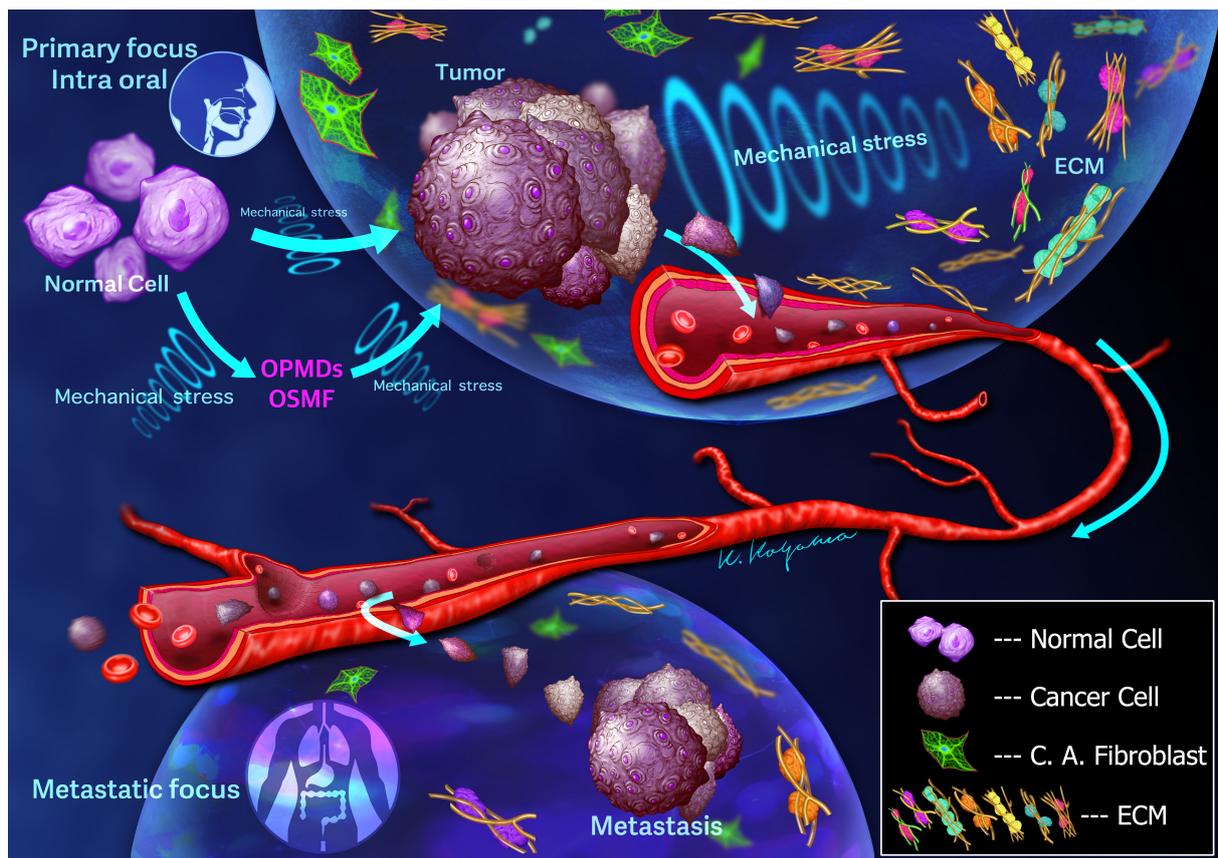


Fig. 1. Schematic illustration depicts the invasion of cancer cells into the vasculature, where they may grow or detach as single cells or clumps and travel through the circulatory system. Flexible movement using blebs is necessary for invasion and metastasis of cancer cells. Both cancer cells and cancer-associated fibroblasts interact with the extracellular matrix (ECM) components, resulting in various biological processes that determine the fate of cancer. Furthermore, not only cancer cells but also normal cells are definitely affected by mechanical stress, leading to the development of oral potentially malignant disorders (OPMDs) and oral submucous fibrosis (OSMF).

formation of metastatic oral cancer cells. Carcinoma cells initiate the metastatic cascade and extend invasive pseudopodia through breaches in the basement membrane—an extracellular matrix macromolecular barrier that underlies epithelial cells and encapsulates blood vessels [71]. A subset of metalloproteinases from the matrix metalloproteinase gene family is consistently upregulated in invasive carcinomas [72,73]. Cancer metastasis involves a sequence of steps, commencing with the invasion of the adjacent normal stroma. Upon penetration of vascular or lymphatic channels, invading cells may either grow, detach as individual cells or clumps, and navigate the circulatory system (Fig. 1). Cancer cells surviving the host's immune defenses, non-immune defenses, and circulation turbulence subsequently arrest within the capillary bed of susceptible organs, undergo extravasation into organ parenchyma, propagate, and initiate metastasis [74].

The genesis of OSMF in a subset of areca chewers might be related to LO, a copper-activated enzyme pivotal in collagen cross-linking and ECM organization [75]. Betel quid (BQ) can impact ECM turnover by regulating fac-

tors like TGF- β 1, plasminogen activator inhibitor-1 (PAI-1), cystatin, LO, tissue inhibitors of metalloproteinases, and metalloproteinases [9]. Components of areca nut extract resulting from BQ chewing may promote tumor invasion and metastasis by stimulating MMP-9 mRNA expression and secretion. This event correlates with ROS, TGF- β 1/Smad-dependent (Smad2), TAK1, and Smad-independent (EGFR, JAK, PI3K/Akt, MEK/ERK) signaling pathways, but not COX activation. These signaling pathways could potentially find application in preventing and treating BQ chewing-related oral cancers and other diseases [76]. In addition, carcinoma-associated fibroblasts (CAFs) are one of the main cellular components of the tumor microenvironment and promote cancer progression by modifying the ECM [77]. Therefore, the target of mechanical stress in the complexes in multiple cells including CAFs in cancer needs to be discussed for future treatment strategies. Our group previously reported that the recurrent oral cancer with spindle cell carcinoma phenotype showed EGFR target reagent resistant CAFs proliferation and cancer progression [78]. Considering that mechanical stress relating-molecules are

potentially activated in patient samples refractory to EGFR-targeted therapy, EGFR inhibitors may temporarily inactivate these molecules, but intrinsic hyperactivation or acquired reactivation of them may confer resistance to EGFR inhibitors in OSCC cells. Therapies targeting mechanical stress-related proteins may be an effective potential therapeutic option in combination with existing therapies [79]. Not only external stimuli but also intra cellular modulation can change cell morphology to obtain cancer cell properties. As described in this review, malignant cancer cells have extremely high motility and can burrow into blood vessels and metastasize to other tissues in the body. At this time, cancer cells actively form large protrusions called blebs in front of them, and by using these as legs, they can move through narrow gaps while significantly changing their shape (Fig. 1). Aoki *et al.* [80] found that within the blebs of expanding cancer cells, the fluidity of the cytoplasm increases significantly, forming soft cytoplasmic regions. Furthermore, authors found that there is a large influx of calcium ions into the expanding blebs, which causes changes in the properties of the cytoplasm. Thus, cells are able to flexibly deform during cell movement by partially changing the softness of their cytoplasm [80]. By verifying the mechanism that changes the fluidity/softness of the cytoplasm, it is expected that it will lead to the development of new treatments that prevent cancer cell metastasis.

In this review, we have also discussed the regulation of mechanical stress and its signaling pathways in OSMF, OPMDs, and cancer cells. Nonetheless, comprehending the underlying mechanisms of each disease in distinct nationalities and populations remains essential. Alterations in tumor structure, particularly arising from cancer cell and matrix stiffness, offer predictive value for human cancer outcomes. Stiffness holds promise for prognosis prediction and may function as a therapeutic target. In the context of solid tumors, targeting such properties through drug intervention during induction chemotherapy to manage tumor hardness and its associated microenvironment, followed by assessing sensitivity, could prove effective. Alternatively, even with the same surgical procedure, a strategy involving the decision to perform a resection with a larger margin could be considered.

Author Contributions

TH contributed to the research design and prepared the manuscript. TH listed previous reports for this review and critically revised the manuscript. TO contributed to the research design and conception of this work and oversaw. TO also prepared the manuscript. YS prepared the manuscript, interpreted the conception and critically revised the manuscript. SA contributed to the research design and prepared the manuscript. SA also listed previous reports for this review and critically revised the manuscript. TN contributed to the preparation of the manuscript. TN also interpreted the conception and criti-

cally revised the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Given his role as Guest Editor, Takehito Ouchi had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Amedeo Amedei.

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