Contemporary Oral Oncology

Biology, Epidemiology, Etiology, and Prevention

Moni Abraham Kuriakose
Editor

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Editor
Moni Abraham Kuriakose
Department of Head and Neck, Plastic and
Reconstructive Surgery
Roswell Park Cancer Institute
Buffalo
USA

ISBN 978-3-319-14910-3 ISBN 978-3-319-14911-0 (eBook)
DOI 10.1007/978-3-319-14911-0

Library of Congress Control Number: 2016955546

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To my parents for igniting the fire for gaining and sharing knowledge
To my teachers and colleagues for keeping that fire burning
To my students for keeping me on my toes
To Rohan and Mili for keeping me grounded
To my patients for enduring our quest for cure
To Maria for being a patient partner in this quest

MAK

Foreword

Writing and editing a comprehensive multivolume text and a reference source on a focused topic is a dream of a life time for scores of academicians, but only a handful are capable of and committed to realize that dream. Dr. Moni Abraham Kuriakose is to be commended to bring that dream to a reality in the field of oral cancer. He has successfully gathered an assembly of world-class leaders from all corners of the globe to contribute to this exhaustive four-volume treatise on the current state of the art and science of oral oncology. The organization and planning of such an in-depth reference source takes deep understanding of the biology of the disease, and mastery in clinical management of the patient. The editor in chief has very carefully selected scholars from the Roswell Park Memorial Institute, coupled with others from North America, Europe, and Australasia, in the specialty of oro-maxillo-facial surgery and oncology, to have a global perspective of the disease. This provides a global perspective from different geographic regions of the world, with diverse patient populations and varied socioeconomic and cultural differences.

Although, the commonly identified etiologic agents for oral cancer are prevalent throughout the world, the biological behavior and natural history of these tumors are different in various regions of the world. For example, the presentation and behavior of oral cancer seen in South Asia is quite different than that in the western world. The authors have very elegantly delved into the biology of these differences and have highlighted the frontiers in research in this area. Similarly, practical issues in the clinical management of patients in diverse socioeconomic regions are discussed to make this a valuable resource for clinicians throughout the world.

This four-volume, in-depth, and exhaustive text presents frontiers in current research in basic sciences and the biological basis of carcinogenesis, tumor progression, metastases, and recurrence. The breadth and depth of the biology of squamous carcinoma covered in the text by global experts is impressive. Equally well covered are the chapters on diagnosis, treatment, operative technical details, and outcomes: both functional and oncologic. Each chapter is well illustrated with photographs, and superb artwork, to convey to the reader the intricate details from biological processes, to surgical techniques. Each and every chapter is accompanied by an endless list of references, to make this a "go to" resource and a reference text on the topic. This opus of oral oncology from molecular signatures to CAD-CAM technology in reconstructive surgery is a one of a kind publication on this subject published in a long time.

The four-volume set in *Contemporary Oral Oncology*, will have a solid place in the libraries of medical schools, postgraduate institutions, Cancer centers, and specialty departments in Universities. It is a wonderful state-of-the-art resource for the trainee as well as the practitioners of oral oncology, to remain current with the topic, and as a ready reference in basic and clinical research as well as day today management of patients. This exhaustive work stands alone in the presentation of biology, diagnosis, clinical care, prevention, and outcomes in oral cancer.

New York, NY, USA

Jatin P. Shah,
MD, PhD(Hon), DSc(Hon), FACS,
FRCS(Hon), FDSRCS(Hon), FRCSDS(Hon), FRACS(Hon)
Professor of Surgery
E W Strong Chair in Head and Neck Oncology
Memorial Sloan Kettering Cancer Center

Preface

Oral oncology is emerging as a distinct discipline. Comprehensive management of oral cancer requires multidisciplinary input of interconnected specialties. Every aspect of the management from diagnosis, treatment, reconstruction, and rehabilitation has biological basis. The biologic understanding of oral cancer and the treatment is changing with time. Understanding and updating developments in each of the related fields are essential to offer the patients the best possible treatment.

This book, in four volumes, is an in-depth reference guide that covers all aspects of the management of oral cancer from a multidisciplinary perspective and on the basis of a strong scientific foundation. Individual volumes are devoted to tumor biology, epidemiology, etiology, and prevention; diagnosis and treatment options; reconstructive surgical techniques; and rehabilitation and supportive care. By integrating current scientific knowledge into a manual for comprehensive care of the oral cavity cancer patient, this book is expected to fill a substantial void in the literature. Further key features are attention to the practical significance of emerging technology and the inclusion of contributions from authors in diverse geographic locations and practice settings in order to ensure that the guidance is of global relevance. The text is supported by ample illustrations and by case studies highlighting important practical issues.

There is lack of a single multidisciplinary comprehensive reference guide in oral oncology. This book is envisioned to fill this substantial void in literature. This book is intended for both trainees and practicing specialists in oral oncology. During my training, clinical practice, and research, I had the opportunity to gain knowledge and skills from different disciplines that includes dentistry, medicine, oral and maxillofacial surgery, general surgery, otolaryngology, plastic surgery, and basic science research spanning three continents. This unique opportunity provided me an insight into the importance of cross-fertilization of ideas from different disciplines and geographic regions. This book is an attempt to impart that principle to the field of oral oncology.

The first volume is dedicated to tumor biology, epidemiology, etiology, emerging role of cancer stem cells, and the prevention of oral cancer. It opens by discussing oral carcinogenesis in general and the role of different carcinogens and human papillomavirus in particular. Global epidemiology and changes in disease prevalence are then addressed. Up-to-date information is provided on emerging cancer

biomarkers, and the biologic basis of personalized therapy is explained. Histopathological features of malignant and premalignant neoplasms and their relevance to management are described. Further chapters focus on the current status of chemoprevention, the management of oral submucous fibrosis, and the value of various diagnostic adjuncts. This volume concludes by critically evaluating the efficacy of oral screening methods.

The second volume deals with diagnosis and management of oral cancer. This volume addresses a range of management issues in oral cancer, from imaging and staging through to the roles of radiation therapy and chemotherapy. Principles of ablative surgery are explained, and neck dissection and sentinel lymph node biopsy techniques are described. Detailed consideration is also given to the management of complications, salvage surgery and re-radiation, the biologic basis of treatment failure, and emerging approaches to overcome treatment resistance. The inclusion of resource-stratified guidelines will meet the needs of practitioners in different geographic regions with varying resources.

The third volume is devoted to the reconstructive surgical techniques used in patients with oral cancer. Following introductory chapters outlining the general principles of reconstructive surgery in the oral cavity and the planning of maxillofacial reconstruction, detailed descriptions of the options and techniques employed in reconstruction of each of the functional subunits are provided. Important technologic advances are also discussed, including image-guided surgery, robotic surgery, and tissue-engineered and prefabricated approaches. Finally, the current status of face transplantation for maxillofacial reconstruction is reviewed.

The last of this four-volume book deals with the most important and often neglected aspect of rehabilitation and supportive care. This volume focuses on the topic of comprehensive rehabilitation and supportive care in oral cancer. The coverage includes the role of maxillofacial prosthodontics, advances in anaplastology techniques, and management of oral mucositis during radiation and chemotherapy. Holistic and supportive care approaches are discussed, and advice is provided on post-therapy surveillance and the use of different measures to assess quality of life. Nutritional evaluation and management and issues relating to healthcare economics are also considered. This volume will be of interest both to practicing specialists and to ancillary service staff involved in the care of oral cancer patients.

This book was authored by leaders in the field from diverse medical disciples and geographic regions. I thank the authors whose expertise and hard work that has distilled a vast body of information into a clear and detailed discussion of various aspects of oral oncology. I would like to express my thanks to the Springer Nature for supporting me in developing this book, to Wilma McHugh for project management and constant support, and to Abha Krishnan and Eswaran Kayalvizhi for the editorial assistance.

I have personally benefitted immensely by the tutelage of many mentors notably Sripathy Rao, Paul Salins, K. Kamalamma, Adrian Sugar, Anwar Perriman, Montague Barker, Paddy Smith, Brian Awry, John Hawksford, Keith Postlethwaite, Leo Stassen, Ian Martin, Andrew Ryan, Collin Edge, Mark DeLacure, Wesley Hicks Jr., Thom Loree, Richard Bankert, and my colleagues at New York University:

Mark DeLacure, Richard Cohen, Robert Glickman, Fang-An Chen; Roswell Park Cancer Institute: Wesley Hicks Jr., Hassan Arshad, David Cohan, Vishal Gupta, Robert Lohman, Wong Moon, Can Ozturk, Cemile Ozturk, Paul Tomljanovich; Amrita Institute of Medical Sciences, Kochi: Subramanya Iyer, Jerry Paul, Sherry Peter, Pramod Subash, Maria Kuriakose; and Mazumdar-Shaw Cancer Center, Bangalore: Vikram Kekatpure, Amritha Suresh, Naveen Hedne, Vijay Pillai, Vinay Kumar, and Praveen Birur. Many of their thoughts will be reflected in this work. I am also indebted to my clinical and research fellows at New York University, Amrita Institute of Medical Science, Mazumdar-Shaw Cancer Center, Roswell Park Cancer Institute, and research associates and doctoral students at Mazumdar-Shaw Center for Translational Research, Bangalore.

Buffalo, NY, USA

Moni Abraham Kuriakose, MD, FDSRCS, FFDRCS, FRCS (Edn), FRCS

Contents

Carcinogenesis and Field Cancerization in Oral Squamous Cell Carcinoma

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Amritha Suresh, Moni Abraham Kuriakose, Simple Mohanta, and Gangotri Siddappa

1.1 Oral Carcinogenesis

Oral carcinogenesis is a multistep, multifocal process initiated as a consequence of carcinogenic insults on the oral mucosa in individuals with genetic susceptibility for oral cancer. The carcinogenic process results in successive molecular changes that lead to dysregulation of cell proliferation, growth, and differentiation. The changes at the genetic and molecular levels ultimately lead to cellular transformation and carcinogenesis. The carcinogenic process in oral cancer, as is the case with majority of other solid tumors, occurs stepwise fashion at molecular, histological, and clinical levels.

Clinically a significant proportion of oral cancers develop as white patch (leukoplakia), mixed white and red patch (speckled leukoplakia), and red patch (erythroplakia). There is higher rate of dysplastic lesions in erythroplakia as compared to leukoplakia. A subset of leukoplakia with verrucous surface morphology called proliferative verrucous leukoplakia has the highest malignant transformation potential. A significant number of oral squamous cell carcinomas develop in clinically normal mucosa. In this scenario it is assumed that the molecular changes of oral carcinogenesis has not lead to changes in the appearance of the oral mucosa. Novel diagnostic adjuncts are being developed to detect these sub-clinical lesions. Therefore clinical appearance does

A. Suresh, PhD • S. Mohanta, PhD • G. Siddappa, PhD (✉)
Integrated Head and Neck Oncology Program, Mazumdar Shaw Center for Translational Research, Mazumdar Shaw Medical Foundation, Bangalore, India
e-mail: amritha.suresh@gmail.com

M.A. Kuriakose, MD, FRCS
Department of Head and Neck, Plastic and Reconstructive Surgery, Roswell Park Cancer Institute, Buffalo, NY, USA

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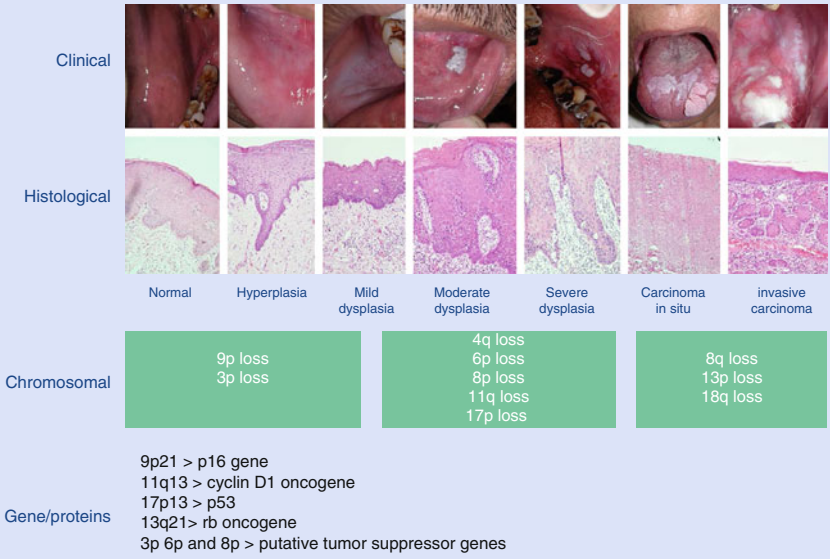
M.A. Kuriakose (ed.), *Contemporary Oral Oncology: Biology, Epidemiology, Etiology, and Prevention*, DOI 10.1007/978-3-319-14911-0_1

not correlates with histology of lesions. A biopsy is essential to make management decision. At the histological level, the disease progresses from epithelial hyperplasia and hyperkeratosis, mild dysplasia, moderate dysplasia, severe dysplasia to carcinoma in situ and invasive carcinoma. It is to be noted that not all the lesions progress linearly on one direction as many dysplastic lesions can reverse to non-dysplastic lesions. On a chromosomal level, some of the early changes are seen at loss of 9p and 3p loci. With dysplastic lesions, loss of 4q, 6p, 8p, 11q, and 17p loci are seen. In invasive carcinoma, 8q, 13p, and 18q loss are seen. Corresponding changes in genes are also observed during malignant transformation. These include p16, cyclin D1, p53, and pRb



1.1.1 Clinical Progression of Oral Cancer

The concept of a two-step carcinogenesis process in the oral mucosa is well established [1]. The initial dysplastic changes lead to the development of premalignant lesions which then develop into carcinoma. The potentially malignant lesions have varying rates of malignant transformation potential, adding to the challenge of an accurate detection of susceptible lesions. However, evidences also exist that point out to the development of oral carcinoma without being preceded by clinically overt premalignant lesions.

1.1.1.1 Potentially Malignant Lesions

The two well-known types of oral premalignant lesions (PMLs) with varying rates of transformation are leukoplakia (2–8 %) and erythroplakia (14–67 %) [2]

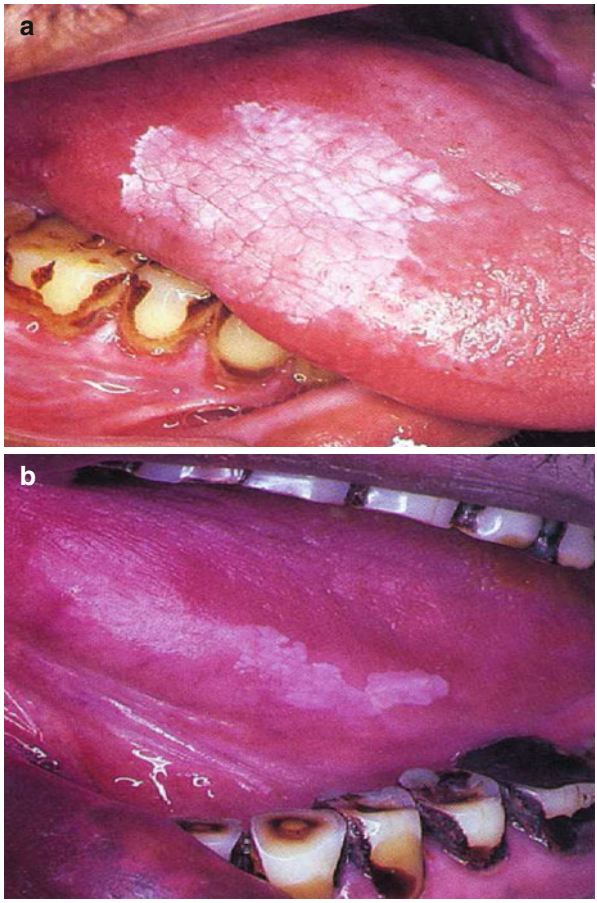


Fig. 1.1 Clinical appearance of leukoplakia (**a**) and erythroplakia (**b**)

(Fig. 1.1a, b). Leukoplakia can be defined as "white patch or plaque" that cannot be characterized clinically or pathologically as any other disease and is not associated with any physical or chemical causative agent except the use of tobacco [3], whereas erythroplakia is a clinical term given for a chronic red mucosal macule that looks similar to leukoplakia, but which cannot be attributed to traumatic, vascular, or inflammatory causes [3, 4]. Nevertheless, erythroplakia is less common than the white precancerous lesions, and on careful observation, they are found to be associated with many early invasive oral carcinomas. A variant of oral leukoplakia was recently described called proliferative verrucous leukoplakia (PVL) with very high prevalence of malignant transformation. PVL can be defined as a progression of white mucosal plaques that virtually always develops into nodular, papillary, or verruciform surface projections and gradually, sometimes rapidly, spreads laterally to cover up large regions of oral mucosa. It was also reported that PVLs have high transformation rate (>70 %) when compared to other potentially malignant lesions [5, 6].

Other types of potentially malignant conditions include oral submucous fibrosis (OSMF) and lichen planus. It was observed that OSMF is strongly associated with the chewing habit (areca nut, betel quid, gutka) and is an irreversible precancerous lesion with a transformation rate of 5 % [7]. It was shown in a study that patients with both oral leukoplakia and oral submucous fibrosis (OSMF) are at higher risk for malignant transformation into oral cancer when compared to the presence of either of these lesions alone, the risk for leukoplakia being the most significant [8]. Lichen planus, an autoimmune disorder of the oral mucosa and skin, typically represented as intertwining, thin strands or streaks of white keratosis (Wickham's striae) with a very low rate of transformation (<1 %) [9, 10].

1.1.2 Histological Progression

The histological neoplastic progression is based on the grade of dysplasia. This is determined by multiple tissue architecture (epithelial stratification, polarity, mitotic figures, keratinization, keratin pearls) and cellular criteria (nuclear/cytoplasmic ratio, nuclear pleomorphism, and size). The hierarchical gradation of histological progression is from normal to hyperplasia, to mild, moderate, and severe dysplasia. This is subsequently followed by carcinoma in situ which ultimately progresses into squamous cell carcinoma.

The major cellular-based changes during progression from normal to hyperplasia, dysplasia, carcinoma in situ, and squamous cell carcinoma include abnormal variation in nuclear size and shape (anisonucleosis and pleomorphism), increased nuclear/cytoplasmic ratio, enlarged nuclei and cells, hyperchromatic nuclei, increased mitotic figures, abnormal mitotic figures (abnormal in shape or location), and increased number and size of nucleoli [11]. These changes evident in the epithelial cells can enable the identification of atypical cells during the process of carcinogenic progression (Table 1.1) [12].

The variability of these changes depends on the grade of dysplasia. Similarly, there are few major architectural/tissue-based changes that can be observed during the progression which include loss of polarity, disordered maturation from basal to squamous cells, increased cellular density, basal cell hyperplasia, dyskeratosis (premature keratinization and keratin pearls deep in the epithelium), bulbous drop-shaped rete pegs, secondary extensions (nodules) on rete tips, and top-to-bottom change of carcinoma in situ [11]. These tissue-based and cellular-based changes involved in each grade of dysplasia are as mentioned below in Table 1.2.

Table 1.1 Groups showing light microscopic and scanning electron microscopic difference in epithelium and epithelial cells [20]

Groups studied	Light microscopic study (difference in epithelium)	Scanning electron microscopic study (difference in epithelial cells)
Normal mucosa	Non-keratinized stratified squamous epithelium	Flat-surfaced cells with equidistant parallel micro ridges
Oral mucosa exposed to tobacco/alcohol	Hyper-para-keratinized stratified squamous epithelium with mild cytological atypia	Irregular and widened micro ridges with numerous pits and absence of honeycomb pattern
Clinically diagnosed leukoplakia	Architectural and cytological changes	Irregularly arranged broad and swollen cells with numerous pits and irregular microvilli projecting over the surface

Table 1.2 Cytological and architectural changes during progression of the disease from normal to hyperplasia, dysplasia, and carcinoma in situ [21]

Grade	Levels involved	Cytological changes	Architectural changes
Hyperplasia	N/A	None	Thickened epithelium Hyperkeratosis Normal maturation Basal cell hyperplasia
Mild	Lower third	Cellular and nuclear pleomorphism Nuclear hyperchromatism	Basal cell hyperplasia
Moderate	Up to middle third	Cell and nuclear pleomorphism Anisocytosis and anisonucleosis Nuclear hyperchromatism Increased and abnormal mitotic figures	Loss of polarity Disordered maturation from basal to squamous cells Increased cellular density Basal cell hyperplasia Bulbous drop-shaped rete pegs
Severe	Up to upper third	Cell and nuclear pleomorphism Anisocytosis and anisonucleosis Nuclear hyperchromatism Increased and abnormal mitotic figures Enlarged nuclei and cells Hyperchromatic nuclei Increased number and size of nucleoli Apoptotic bodies	Disordered maturation from basal to squamous cells Increased cellular density Basal cell hyperplasia Dyskeratosis (premature keratinization and keratin pearls deep in epithelium) Bulbous drop-shaped rete pegs Secondary extensions (nodules) on rete tips Acantholysis
Carcinoma in situ	Full thickness	All changes may be present	Top-to-bottom change Loss of stratification

1.1.3 Molecular Model of Progression

Knudson two-hit hypothesis: Chromosomes are represented in pairs in the normal cells, one inherited from the mother and the other from the father. All the genes have representation on both the chromosomes of a pair in the form of alleles. The first hit of carcinogenic insult can cause loss or mutation of an allele in one of the chromosomes. The first hit is usually thought of as a point mutation that inactivates one copy of a tumor suppressor gene, such as Rb1. The individual does not develop cancer at this point because the remaining tumor suppressor gene allele on the other locus is still functioning normally. This loss of heterozygosity is common in many cancers. Continued carcinogenic insult can lead to loss of the remaining normal gene (second hit) leading to loss of function of that gene. If this happens to be, a tumor suppressor gene (e.g., p53), it can affect DNA repair mechanism and the integrity of the entire genome leading to development of cancer.

In hereditary cancers (e.g., xeroderma pigmentosa where nucleotide excision repair enzyme gene is defective), the individual is born with one mutated gene. Carcinogenic insult can cause deletion of the normal functioning gene leading to the development of cancer. This two-step process of genetic basis for carcinogenesis is called Knudson two-hit hypothesis.

The early molecular models developed based on marker correlation with stepwise histological progression changes [12–14] indicated that specific molecular changes associated with each stage of histology. Loss of heterozygosity (LOH) [see definition] one of the earliest chromosomal abnormalities to be associated with cancer at specific sites correlated with histological progression. LOH at 3p and 9p along with allelic instability at both the loci is one of the biomarkers identified as the initial steps of malignant transformation [15–19]. LOH at 9p with TP53 mutation was also shown to be associated with malignant transformation and can be similarly used as a biomarker for malignant transformation prediction [20]. Recent studies have also reported that LOH at 15 microsatellite markers [3p, 9p, 17p, 8p, 13q and 18q] was observed frequently in histologically higher-grade lesions (moderate or severe dysplasia) and in lower-grade lesions (mild dysplasia) when there is a high proliferation rate [21]. It was also shown that LOH at 4q, 8p, 11q, and 13q was significantly associated with presentation of dysplastic lesions along with marginal significance for LOH at 17p, whereas LOH at 4q, 8p and 17q were associated with hyperplasia, 11q LOH showing a marginally signficant association [18]. Studies over the past decade have also identified various molecular players associated in the initial neoplastic development and subsequent progression to carcinoma.

In addition to these changes that were documented as essential during the stepwise progression of oral carcinogenesis, studies have identified many molecular players that contribute toward the initiation and progression of oral cancer.

1.1.4 Biomarkers of Oral Carcinogenesis

1.1.4.1 Tumor Suppressor Genes (TSG)

Deregulation of the tumor suppressor gene family is one of the earliest events in initiation of tumorigenesis. Molecular changes in p53/Rb and the p16/pRb/cyclin D1 pathway are known to lead to acquisition of dysplastic characteristics [22]. Correlation with patients at various stages of oral cancer progression has been the primary mode of understanding the role of markers during the process. Multiple studies have shown that the expression of suprabasal p53 is associated with different grades of dysplasia [23–25]. CDK1 (p21) is another TSG that correlated with early dysplastic progression with the gene showing a significant and progressive increase in expression from mild (3 %) to moderate (50 %) and severe dysplasia (64 %) [26]. P27 on the other hand showed a positive suprabasal staining pattern in normal and mild dysplasia that became less apparent with increasing degrees of dysplasia [26–28]. Among the Rb family of TSGs, a significant loss of Rb and p16 levels was reported at the transition from hyperplasia to dysplasia [22, 26, 29, 30].

Progression of dysplastic lesions to carcinoma mostly involves a further increase in cell proliferation accompanied by an increase in properties of migration and invasion, ultimately leading to metastasis. Multivariate analyses in different studies has shown that although deregulation of p16/pRb/Cyclin D1 pathway is an early event in dysplasia development, both pRb and p53 pathways are associated with malignant transformation and adverse prognosis in oral cancer [22, 31]. Genetic alterations identified in the retinoblastoma (Rb) family members, pRb, pRb2/p130, and p107, are reported to be involved in growth arrest, apoptosis, differentiation, and angiogenesis that may act as significant factors for pathogenesis and progression of tumor in various cancers and, hence, may be useful for assessing the risk in cancer patients. Expression of pRb2/p130 may be inversely correlated with malignancy of oral dysplastic lesions and, hence, can be used as an indicator for progression [32, 33]. PTEN, a candidate tumor suppressor gene located at 10q23.3, might also play an important role since lack of PTEN expression is an independent prognostic indicator for clinical outcome, as observed in patients with tongue cancer [34].

1.1.4.2 Cell Cycle and Proliferation Markers

Cell cycle deregulation is one of the major means by which malignant transformation is affected; the pathways and molecules involved in this process hence show differential regulation during the various stages of oral carcinogenesis. It was shown that a combination of elevated expression of cyclin-dependent kinases (cyclin D1, cyclin E, CDK2) and loss of epigenetic markers [p12 (DOC-1), p16 (INK4A), p27

(KIP1)] may contribute to the multistep nature of oral carcinogenesis [26]. p27Kip1, a member of the CIP/KIP family of CDK inhibitors that negatively regulates cyclin-cdk complexes, was found to show reduced expression and was associated with increased cell proliferation, although other changes might contribute to altered cell kinetics during carcinogenesis [27].

Minichromosome maintenance protein (MCM2-7) is essential for eukaryotic replication initiation and along with another cell cycle protein, Geminin, are suggested to be novel biomarkers of growth and proliferation in oral epithelial dysplasia [35, 36]. Other cell cycle proteins such Cdc6 were also overexpressed, with the expression correlating to the development and metastasis of oral cancer suggesting that it can be a molecular marker for early diagnosis and prognosis prediction [36].

Suprabasal expression of Ki-67, an indicator of proliferation, was directly associated with the presence and severity of oral dysplasia [37, 38]. It was reported that oral dysplasia is characterized by lower cell proliferation and a higher frequency of cell death when compared to SCC, and moreover, several indices combining the expression of multiple markers are known to be indicators of dysplastic development. High labeling indices (LI) of minichromosome maintenance 2 (MCM2) and p53 and lower LI of p21 are suggested to be helpful in the prediction of malignant transformation of oral dysplasia and also as a biomarker of proliferating cells [39].

1.1.4.3 Angiogenesis and Metastatic Markers

Angiogenesis is vital to the malignant transformation process; molecules that facilitate the process are thus possible indicators of oral cancer progression. Studies in premalignant lesions have reported Willebrand factor along with p53 to be associated with oral carcinogenesis [40]. Microvascular density (MVD), an indirect marker of neo-angiogenesis, as detected by markers CD31, CD34, and CD105, is known to be significantly associated with different grades of dysplasia [41–43]. Studies have also associated the markers of angiogenesis such as VEGF with the late progression of oral cancer, recurrence, and metastasis rather than the early stages [44, 45].

Tumor protein 63, a p53 homolog, is highly expressed in the nuclei of basal regenerative cells and was commonly upregulated in HNSCC and subsequently resulting in the increased expression of downstream molecules such as MMP14 and LAGLS1, motility-related molecules indicating its efficacy to determine potential metastatic tumors [46, 47]. The increase in p63 and CD105 expression has also been correlated with a concomitant loss of membranous E-cadherin indicating an association with increased EMT behavior [48]. Ezrin, a member of the ERM protein family, plays key roles in cell structure, organization, adhesion, and migration. The Akt/Ezrin Tyr353/NF-κB is known to regulate EGF-induced EMT and metastasis in tongue cancer; EZRIN is suggested to be a therapeutic target to reverse EMT and prevent progression [49].

1.1.4.4 Cytokeratins

Cytokeratins, essential for the maintenance of the cytoskeletal assembly, are found to be extensively overexpressed in HNSCC [50]. The expression pattern of cytokeratin filaments in the epithelium was found to be directly dependent on the type

and differentiation pattern of tumors [51]. CK-10/CK-11 and involucrin that are normally present in terminally differentiating keratinocytes showed strong correlation with the differentiation status of cells: high expression in non-dysplastic hyperplastic epithelium as compared to normal, dysplastic, and neoplastic epithelium. These proteins were also found to be inversely correlated with various grades of dysplasia suggesting that these proteins may be useful biomarkers for epithelial carcinogenesis [52].

1.1.4.5 miRNA Markers

miRNAs are now increasingly implicated in various aspects of carcinogenesis; studies in oral cancer have also revealed their role in malignant transformation. miR-21, miR-181b, and miR-345 were found to be consistently increasing in expression with the increase in severity of the premalignant lesion. Upregulation of miR-181 in OSCC during its progression from leukoplakia to dysplasia to invasive carcinoma was correlated with lymph node metastasis, vascular invasion, and poor survival since upregulation might enhance migration [53, 54]. miRNA markers that are differentially expressed in tissue and saliva with concordant fold levels can be used for monitoring of potential relapse or malignant transformation in oral cancers [49, 55–57].

hTERT, the human telomerase reverse transcriptase, a component of the Telomerase complex, is known to have an elevated expression profile in oral cancers as compared to the normal oral mucosa. Other studies have also shown that this increased expression of hTERT protein was found to be an early event in oral carcinogenesis, and the amount of cytoplasmic or nuclear expression of hTERT was an accurate indicator of progression, recurrence, and prognosis in OSCC [58–62].

Studies have shown that osteopontin (OPN), a secreted, chemokine like protein, can be used as a prognostic marker for OSCC and not for progression since the expression in PMLs was not in accordance with their histological grading and the intensity of expression was also similar to that seen in normal epithelium [63]. Expression of Nuclear factor KB (NF-KB) and Cyclooxygenase 2 (COX-2) proteins, known to be regulated by Osteopontin were found to increase with histological progression of the disease (normal to leukoplakia to carcinoma). It is also reported that NFKB shows a negative correlation in tumor-surgical margin-to extra margin, with COX-2 showing a parallel expression. These studies suggest that NFKB might be involved in the later stages of acquisition of malignant phenotype in oral carcinogenesis while COX-2 may be involved at the early stages [64, 65].

EGFR is a cell surface receptor to which ligands such as epithelial growth factor (EGF) bind. Once activated, it undergoes a fully reversible dimerization to form a homodimer [66]. Deregulated mutant EGFR overexpression was observed in majority of patients with HNSCC and is reported to enhance tumorigenic capabilities [67]. An increased copy number in EGFR gene can be used for the prediction of

malignant transformation in oral premalignant lesions [68]. In oral premalignant lesions, with the expression being higher in high risk lesions.

Claudins, normally expressed in a reticular pattern up to the prickle layer in normal mucosal epithelium, are directly correlated with the grade of tumors and vascular infiltration and inversely correlate with recurrence; Claudin 7, one of the member of the family reported to be a poor prognosticator in Oral cancer [69]. Melanoma-associated antigen-A (MAGE-A), an antigen restricted to malignant cells, can be used as a marker in high-risk patients for an accurate estimation of potential malignant transformation of premalignant lesions [70]. The advanced oxidation protein products (AOPP) obtained from different oxidation patterns were known to produce of either NO or H_2O_2 which leads to the generation of different types of reactive oxygen species that set a cascade of reactions with a potential to damage cellular micromolecules eventually turning out into frank OSCC [71]. Some of the other markers that are known to be associated with early dysplastic progression are the WW-domain-containing oxidoreductase (WWOX) with >35 % of the dysplastic lesions showing altered transcript and protein levels. A combined expression of stromelysin and Ets-1 was shown to be predictive of transition to a precancerous stage with high statistical significance [72].

Oncoprotein Bcl-2 regulates programmed cell death by allowing tumor cells to escape apoptosis and was found to be overexpressed in OSCC as compared to premalignant lesions suggesting its presence in the early stages of carcinogenesis [73]. It was shown that Bax and Bcl-X along with p53 were expressed early, and Bcl-2 and MDM-2 showed sporadic expression in the development of oral premalignant and malignant disease suggesting that protein regulation of apoptosis may be altered during the development of OSCC [23]. An inverse relationship was found between Bcl-2/Bax ratio and apoptosis from normal oral epithelium to severe dysplasia indicating that suppression of Bcl-2 may have a role in oral tumorigenesis [74].

Podoplanin, a mucin-like transmembrane glycoprotein specifically overexpressed in lymphatic endothelial cells, was found to be expressed in hyperplastic and dysplastic areas adjacent to primary tumors indicating that its abnormal expression occurs early in oral tumorigenesis [75]. The subcellular localization of the nuclear S100A7 gene, the calcium binding protein, was found to be expressed in early stages of oral premalignant lesions and was known to be a potential determinant for transformation of oral premalignant lesions and recurrence in HNSCC [76].

1.1.5 Cancer Stem Cells in Oral Carcinogenesis

At normal physiologic condition, stem cells localized at the basal layers are in a tightly regulated quiescent state. These cells undergoes asymmetric cell division with one daughter cell remaining as stem cell and the other as differentiated cells that maintain the epithelial integrity. After epithelial injury, stem cells lose its

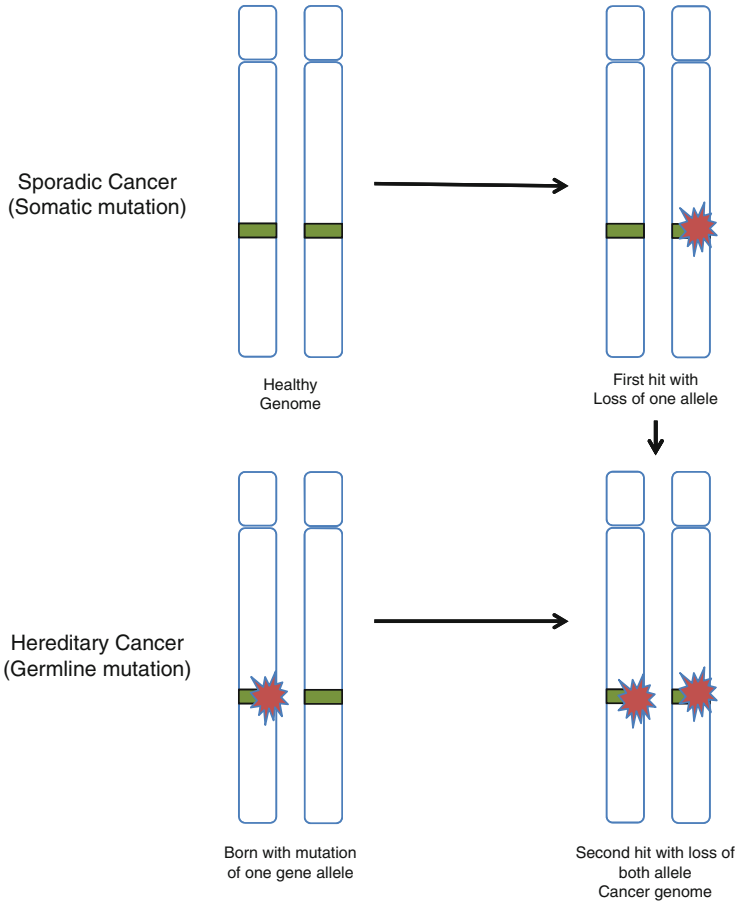


Fig. 1.2 Diagrammatic representation of Knudson two-hit hypothesis

inhibitory signal transiently and produce differentiated cells to repair the wound (Fig. 1.2). Since stem cells are the only long-term resident cells in an epithelium, they are likely to be the target of carcinogenic stimuli. By acquiring series of genetic and epigenetic changes, these stem cells transform into cancer stem cells (CSCs). Another view on origin of CSC is that they develop by dedifferentiation of tumorigenic epithelial cells (Fig. 1.3). In addition to the properties of normal stem cells, it acquires several other characteristics that make them resistant to inhibitory growth signals (Fig. 1.2). These include (a) self-sufficient growth signaling, (b) antigrowth signaling insensitivity, (c) evasion of apoptosis, (d) unlimited replication potential, (e) sustained angiogenesis, (f) and tissue invasion and metastasis [77–79].

Like normal stem cells, CSC are suggested to reside in a specific niche which is constituted mostly by the endothelial cells and the fibroblasts. The CSC-niche cross talk, though not extensively investigated in head and neck cancer, is suggested to be

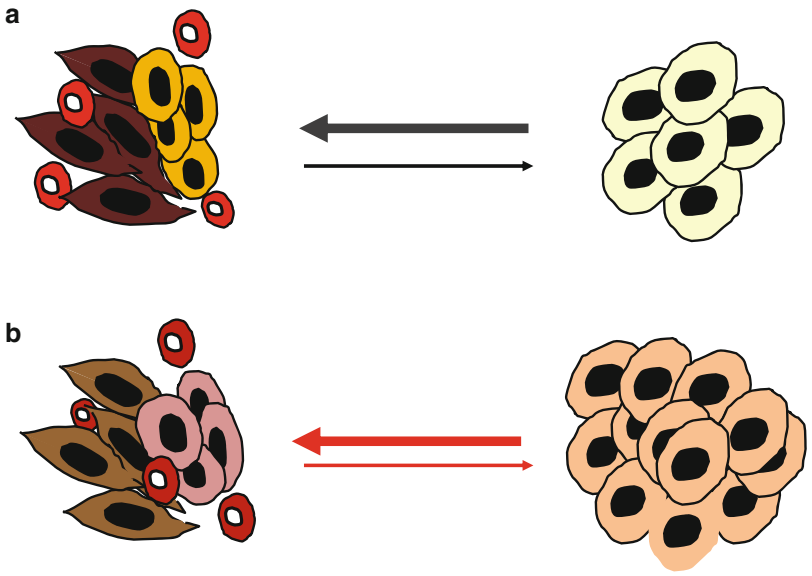


Fig. 1.3 (**a**) Stem cell niche is required for the maintenance of normal epithelial integrity, where the stem cells that are normally in quiescent stage transiently get activated to produce differentiated cells. (**b**) Cancer stem cells on the other hand have lost its negative feedback and are always on a active state

orchestrated by multiple pathways such as the TGFB1 signaling, SDF-1/CXCR4 axis and NPTCH1 signaling. Evidence does suggest that this cross-talk can induce CSC-like properties in the cancerous epithelial cells and/or initiate the development of cancer-associated fibroblasts/endothelial cells through secreted cytokines (eg. CXCL12, TGFB1) and their receptors (eg. CXCR4). The niche can hence play a major role in the carcinogenic process (Figs. 1.3, 1.4 and 1.5).

The cancer stem cell concept in oral carcinogenesis has been supported by the identification of markers that are associated with the early stages of oral cancer development. OCT-4, a protein encoded by the POU5F1 gene, was associated with worse survival rates, and low expression leads to loss of pluripotency [80, 81]. It was also found that ectopic expression of OCT-4 leads to dysplasia in adult mice tissues [82, 83]. Oral premalignant cells also show upregulation of EMMPRIN (CD147) when compared to normal oral epithelial cells. The expression correlates with the degree of dysplasia suggesting that overexpression of EMMPRIN occurs at a very early stage of oral carcinogenesis [84].

Aldehyde dehydrogenase 1 (ALDH 1), an intracellular enzyme, has been a cancer stem cell marker with its expression being higher in OSCC than normal mucosa [2, 85, 86]. ALDH1 and CD133 were also shown to serve as predictors in the identification of oral leukoplakia susceptible to development of oral cancer [87]. Expression of Nanog and Nestin and concurrent levels of OCT4 and SOX-2 were

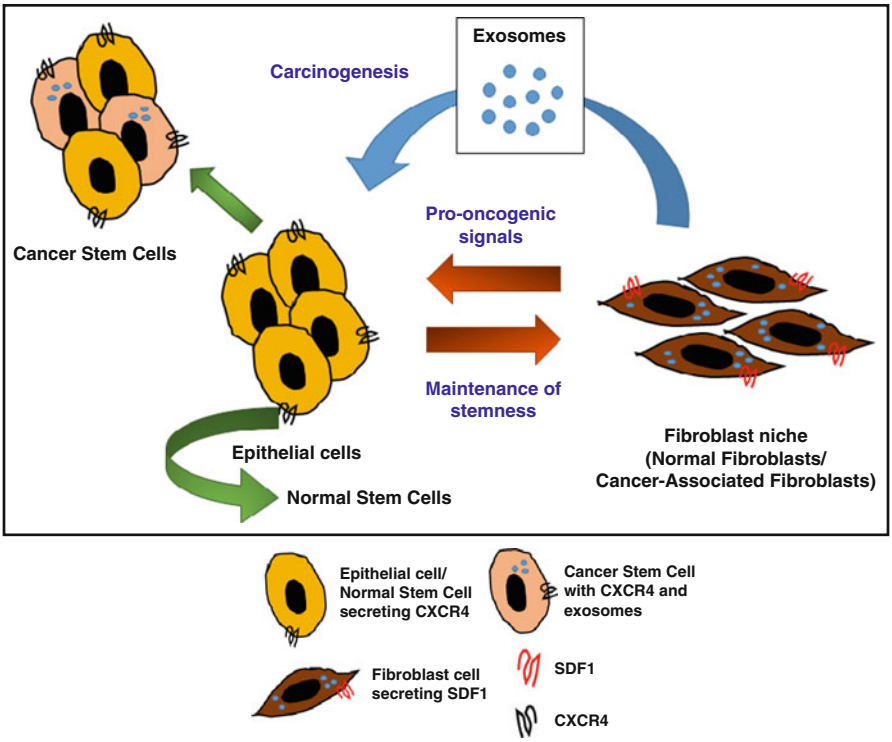


Fig. 1.4 In normal physiologic state, the epithelial stem cells homeostasis is maintained with its interaction with fibroblasts and endothelial cells. In cancer, the fibroblasts develop special features to acquire the phenotype of cancer-associated fibroblasts. The cross talk between cancer stem cells and cancer-associated fibroblasts is explained with the SDF-1/CXCR4 pathway as an example; CXCR4 being expressed on the Cancer stem cells and the SDF-1 being secreted by the niche

associated with low survival rates, aggressive growth, metastasis, and poor prognosis [88, 89]. CD44, a marker for OSCC stem cells, is known to be capable of inducing metastatic properties in nonmetastatic tumor cells [78, 90, 91]. Studies have also shown a gradual increase in the expression of the stem cells markers; CD133 and Musashi-1 observed from normal to dysplasia to carcinoma as well as in advanced and poorly differentiated tumors suggest the involvement of these proteins in oral carcinogenesis [92].

1.1.6 Markers of Oral Carcinogenesis: Implications in Early Detection and Chemoprevention

Early diagnosis is one of the major strategies that can help toward downstaging the disease at presentation and thereby improving survival rates in oral cancer. Advances in the molecular understanding of oral carcinogenesis thus can lead to

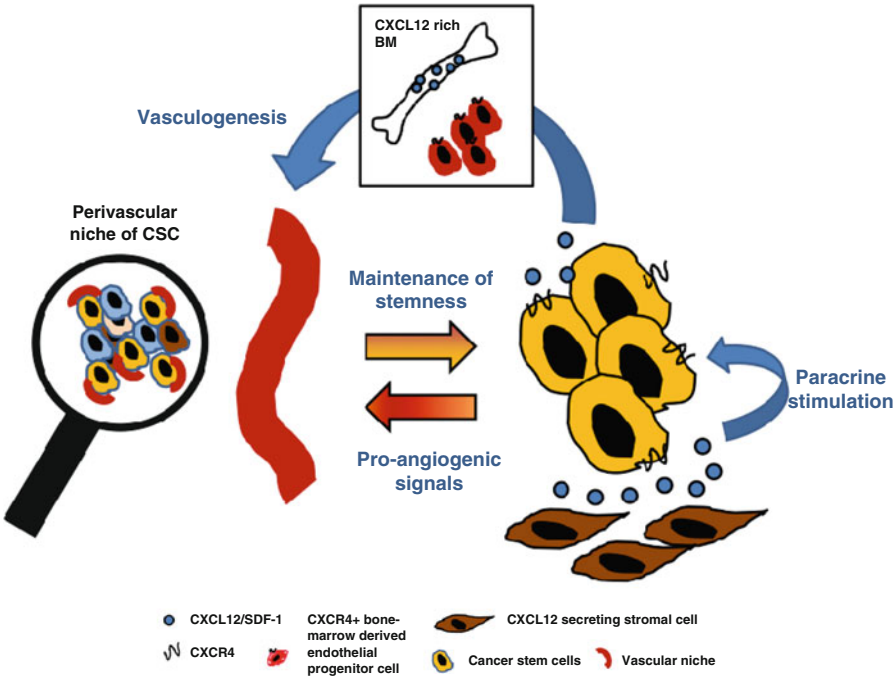


Fig. 1.5 The possible components and interaction of cancer stem cell niche

the identification of potential biomarkers that can be possible candidates for diagnosis. Nevertheless, the markers that have been identified have not been validated for use as diagnosis markers with confirmation by biopsy and subsequent histology being the gold standard. Lectin-based molecular imaging methods utilizing differences in glycosylation have indicated their utility as a diagnostic method [93]. Ongoing studies have also identified markers in saliva and serum that can be assessed for their utility for early detection [94–96]. However, extensive prospective validation studies are mandatory if these markers are to be applied in clinical practice.

Markers of oral carcinogenesis also pave the way to assess novel targets for chemoprevention, the primary strategy that can work toward improving the survival rates of the disease. Several molecules have been tested for their anti-tumorigenic activity in oral cancer. Studies using retinoids, known to differentiate cancer stem cells, showed an average 50 % response in patients with oral leukoplakia [97, 98]. Curcumin that acts on the NFkB pathway is also known to have chemopreventive characteristics as observed in studies on multiple solid tumors including oral cancer [99–101]. Nonetheless, studies are warranted to evaluate the efficacy of targeting the known markers of oral carcinogenesis toward chemoprevention and to also identify other potential novel targets.

1.2 Field Cancerization in Oral Cancer

Incidence of second primary tumors in oral cancer (10–30 % of the cases) occurring at the primary site, despite a complete resection of primary tumor, remains one of the key and challenging issues associated with oral cancer pathogenesis. Clinical studies indicate that "transformed cells" with the ability to initiate new tumors do exist in a histologically normal field surrounding the primary tumor. "Field cancerization," a term coined by Slaughter in 1953, proposes that adjacent normal tissue of tumor harbor certain preneoplastic genetic fingerprints which can eventually lead to local recurrence or second primary tumors, depending on the duration within which the tumor develops. Slaughter and his group based the concept on the following evidences: (1) oral cancer develops in multifocal areas of precancerous changes due to a prolonged and widespread exposure to carcinogens, (2) "abnormal" tissue surrounds the tumor, (3) oral cancer often consists of multiple independent lesions that sometimes coalesce, and (4) the persistence of abnormal tissue after surgery may explain the formation of second primary tumors and local recurrences [102].

It is well known that the onset of carcinogenesis begins long before the clinical detection of the cancerous lesions in the tissue. The detection of morphological changes of cancerous origin occurs at a much later stage during carcinogenesis. Adjacent mucosa surrounding the tumor, though histologically normal, has been shown to have precancerous changes, and these modifications have been suggested to be the cause for the formation of second primary tumors and local recurrence [103, 104], which subsequently lead to poor survival and an increase in mortality rate. Histologically normal cells thus can also harbor the tools and means for cancer formation; most of the studies have proven field cancerization to be one of the reasons behind the recurrence of the disease in the primary as well as at secondary locations. The concept drives the notion of precancerous cells replacing the normal epithelia, making them prone to the genetic and epigenetic changes for tumor formation [105]. Most of the reports provide evidences toward the role of multiple molecular alterations (mutations in oncogenes, loss of heterozygosity, genomic instability and microsatellite alterations, and TSG along with deregulation of the telomerase activity) in field cancerization [147].

1.2.1 Cellular Basis of Field Cancerization

The cellular basis of field cancerization is explained by two main schools of thought: polyclonal mode and the monoclonal mode of origin. Although the complete basis of these models is yet to be established, existing evidences do point out to both these theories being plausible. The classical model for the origin of field cancerization is the "polyclonal model" suggesting that the multifocal carcinomas developing in the region are of independent origin through mutations occurring in multiple sites of the epithelium due to continuous carcinogen exposure [106]. The tumors, thus originating, though are in the adjacent fields, will be genetically

different and are hence polyclonal. Studies in pancreatic and colon cancers suggest a polyclonal origin for the multiple lesions that develop in a patient owing to the distinct K-Ras mutations observed in each lesion [107, 108]. An initial study in head and neck cancer by profiling of p53 and its downstream proteins indicated that simultaneous, preinvasive and invasive lesions in patients showed distinct molecular profile [109, 110].

The monoclonal origin of the field wherein the lesions share a common clonal origin and develop due to migration of the cells from the initial lesion is the second concept of cancerization. Experimental evidences do point out to the feasibility of this model also in bladder cancer, though the underlying basis is not delineated [107, 111]. In order to explain the possible mechanisms driving this concept, three theories have been postulated (Fig. 1.3). *The first theory* suggests that tumor cells or tumor progenitor cells migrate through the submucosa to another site (intraepithelial migration). *The second theory* implies that cells shed into the lumen of an organ (primary tumor site) form the tumor in a secondary site. *The third and the final proposed theory* is based on findings that the genetically altered field in the epithelium originates from clonally related neoplastic lesions that develop *via* lateral spreading in same or adjacent anatomical areas. The final theory also justifies the presence of the patch-field model, wherein a large area of normal mucosa is replaced by genetically altered preneoplastic cells awaiting the second hit to progress to tumorigenic state (Figs. 1.6, 1.7) [103, 104, 106, 112–115].

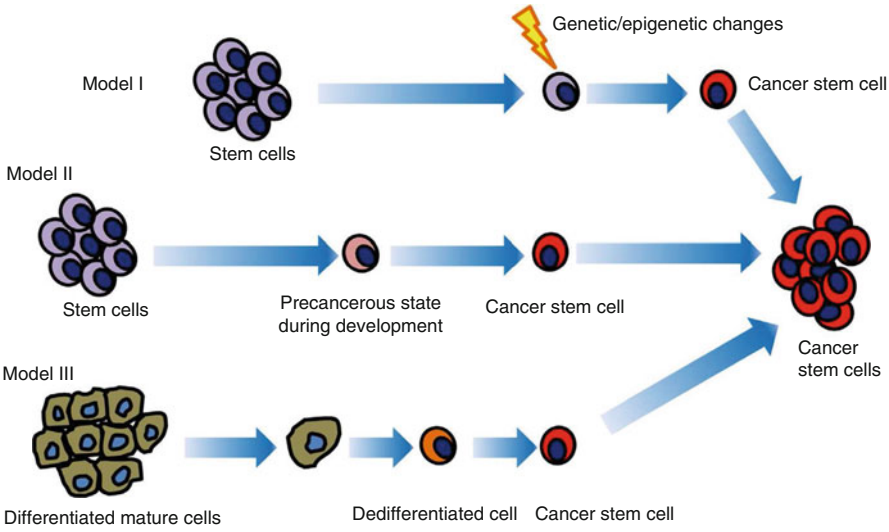


Fig. 1.6 Three possible mechanisms of development of cancer stem cells (Need to get reprint approval from Mohanta et al., 2015 [154])

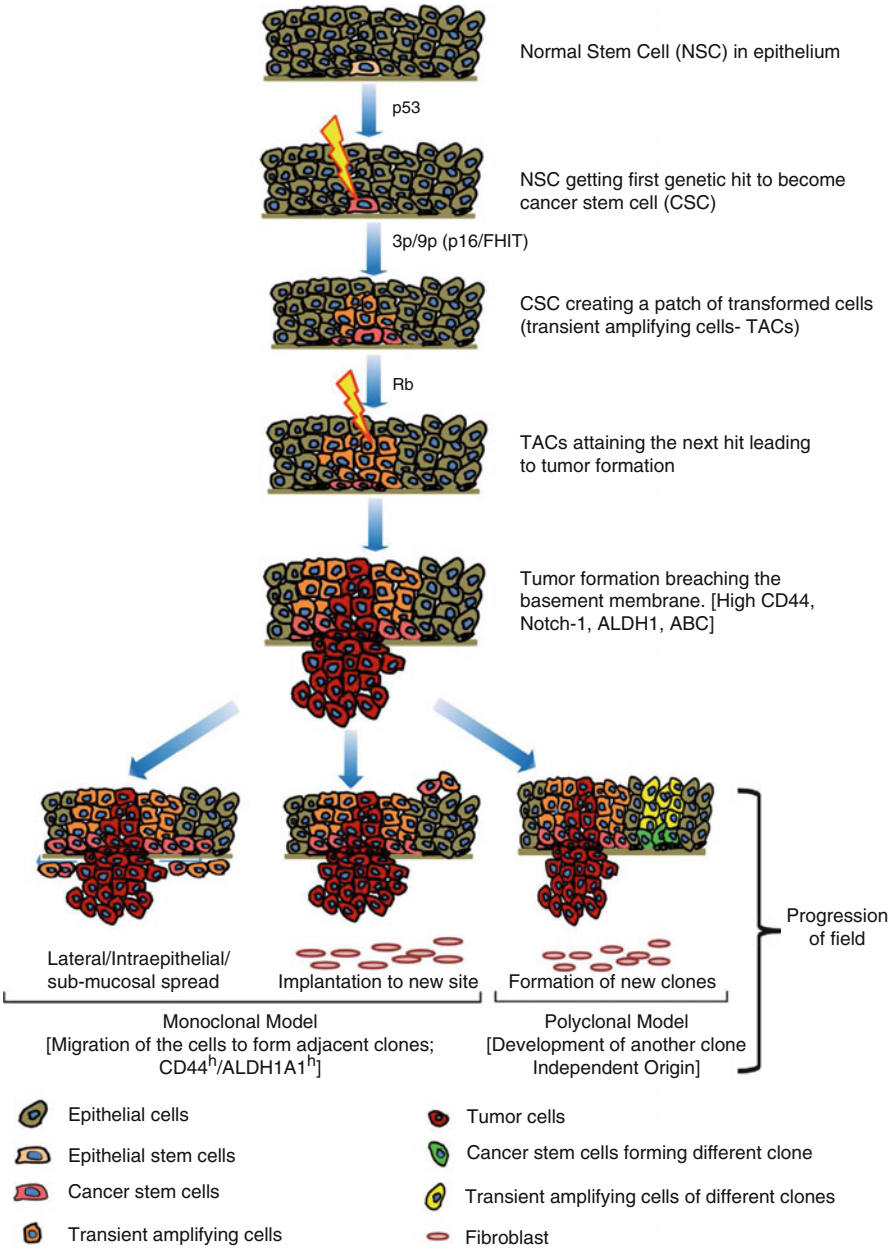


Fig. 1.7 Possible mechanisms of development of field cancerization (Need to get reprint approval from Mohanta et al., 2015 [154])

1.2.2 Molecular Models of Field Cancerization

Multiple models of genetic abnormalities underlying field cancerization have been proposed based on the experimental evidences accumulated down the decades. An initial model proposed by Brakhuis et al. in 2003 [116] is based on the genetic alterations associated with the stepwise histological progression observed in carcinogenesis of HNSCC. The model proposed that the transformation of a normal epithelium to a cancerous one initiates TP53 (17p) mutations in cells which ultimately lead to the development of a patch, consisting of a clonal unit of *these* cells. Subsequently, the patch converts to a field, which is an epithelial lesion consisting of cells with successive cancer-related genetic alterations. This field eventually replaces the normal tissue, and during field progression, additional genetic mutations occur in chromosomes 3p, 9p, 8p, and 18q. With the mutation in 11q12, the field is suggested to transform to a carcinoma *in situ*. During field progression, additional genetic mutations occur in chromosomes 3p, 9p, 8p, and 18q. With the mutation in 11q12, the field is suggested to transform to carcinoma in situ.

Califano et al. have also described the genetic progression model of field cancerization in head and neck cancer. According to this model, the transformation of the normal mucosa is initiated by hits to the 9p region leading to the development of benign hyperplasia. This lesion then further progresses to dysplasia by successive mutations in the 3p and 17p region, with the modifications in 17p region suggested to drive the development of the first patch of mutated neoplastic cells. The patch further expands into the field, which then transforms to cancer with mutation in 11q and 13q chromosomes (18) (Fig. 1.7).

1.2.3 Biomarkers of Field Cancerization

Studies to understand the molecular basis of field cancerization have led to the identification of number of biomarkers that can, ideally, determine the abnormal "field" of transformed cells that are either inherited or arise due to continuous and sustained carcinogenic assault. Detection of these markers in the histologically normal mucosa surrounding the primary tumor is suggested to be indicative of the extent of the field. Molecular markers such as loss of heterozygosity (LOH), microsatellite alterations, telomerase activity, chromosomal instability, and mutation in TP53 gene are some of the established means to distinguish and characterize these resident cells of the "field" that develop during the cancerization process [112, 114, 115, 117–119].

1.2.3.1 TP53 Mutations

p53 overexpression is a common event in head and neck cancers with the protein being involved in the maintenance of cellular integrity caused due to DNA damage. Suppression or alteration of p53 pathway is known to lead to genomic instability and trigger carcinogenesis in head and neck cancer [120]. Assessment of the TP53 status in adjacent normal mucosa provides evidence toward it being one of the early changes that initiate the process of cancerization. Study on patients with oral cancer

showed the presence of p53 mutations in the surgical margins correlated with their clinical outcome [121–123]. On the basis of p53 mutations, Brakhuis et al. classified the tumor as primary tumor, second primary tumor, and recurrent tumor [116, 124] indicating that molecular changes in p53 can be indicative of the clonal origin of the tumor. Mutated p53 was thus considered indicative of molecularly "premalignant cells" in a histologically normal oral mucosa [125].

1.2.3.2 Loss of Heterozygosity

Loss of heterozygosity (LOH), indicating the loss of allelic material adjacent to microsatellite markers, is another marker used to study the clonality of premalignant lesions in the adjacent normal mucosa of the tumor. LOH at different chromosomal locations is known to be an established marker of field cancerization in the normal mucosa. Short tandem repeat (STR) markers specific to the regions 3p12, 3p14, 3p21, 9p21, 9p22, 17p13, and 13q14 have been used to identify molecularly abnormal cells in the tumor-adjacent mucosa. Among these markers, evidences in oral cancer show that LOH at 9p21 is detected in histologically normal mucosa, while changes at 3p accompanied dysplastic changes [126]. Abnormalities at 3p and 9p along with 17p have also been used to distinguish the clonality between the multiple invasive and preinvasive lesions in patients with oral cancer [127].

1.2.3.3 Telomerase

Telomerase levels are enhanced in transformed cells as an attempt to achieve immortalization; the presence of this enzyme in the tumor-adjacent mucosa can also be a relevant marker of cells that are transformed at the molecular level. Studies of telomerase activity in sample cohorts that included adjacent mucosa precancerous and cancerous lesions showed high enzyme activity in 30–70 % of normal tissue [61, 128]. This was suggested to be due to the increased tobacco usage in the patient cohorts further emphasizing that concept of the "abnormal field." The assessment of telomerase status by the TRAP assay (telomerase repeat amplification protocol) in the normal mucosa of oral cancer also showed increased levels in the sample that was predictive of recurrence [129]. Similar studies in cancers of other sites such as breast tumor also showed the presence of hTERT expression in histologically normal tissue [122].

1.2.3.4 Ploidy Analysis

Ploidy analysis, which documents the DNA content in the cells, has also been used as a technique to detect abnormal cells in the tumor-adjacent mucosa. Studies in the oral mucosa of the hamster cheek model, have reported that tissues with no atypical dysplastic changes, have been identified to have abnormal DNA content indicative of the cancerization process [130, 131]. Chromosomal polysomy in various grades of dysplasia was also indicative of field cancerization in patients with head and neck cancer [132]. Evaluation of the DNA index (DI) quantifying the diploid, aneuploid status of the cells in oral premalignant and the normal-appearing mucosa has been identified as a highly significant risk factor [133, 134]. Multiple genomic aberrations

at 20q13, 7p22, 11p15, and 16p13 were also identified to be common between the non-dysplastic mucosa and the dysplastic oral lesions [135, 136]. Another study also pointed out that abnormal changes at chromosomes 7 and 17 were significantly different between tumor-adjacent and tumor-distant mucosae, which were also observed in increasing frequency in the different grades of dysplasia [137, 138].

1.2.3.5 Angiogenesis Markers

Nuclear organizer regions and subepithelial vascularization in the tumor-adjacent mucosa are considered accurate markers of abnormal alterations that precede the histological changes [139]. Gazzar et al. have also reported significantly higher vascularity index in tumor-adjacent normal oral mucosa as compared to the mucosa of non-cancer patient. Vascularization as detected by CD31 and VEGF expression, has also been detected in the normal mucosa along with the dysplastic and the non-dysplastic premalignant lesions [42, 140].

1.2.3.6 Other Markers

Studies to assess the molecular changes that characterize the normal, tumor-adjacent mucosa and thereby indicative of field cancerization have identified several other markers. Expression of cytokeratins (CK19, 8/18, 19), MMPs (MMP 9), and growth factors (EGFR, TGF) in adjacent normal mucosa of the tumor have been identified as markers of field cancerization [141, 142]. Expression patterns of MIB1 and Cyclin D1 were significant for determining the field cancerization [143]. Dysregulated expression of adhesion molecules such as CD44, cadherin, and β-catenin is also suggested to be indicative of neoplastic progression in the tumor-adjacent mucosa [144].

1.2.4 Cancer Stem Cells in Field Cancerization

Cancer stem cells (CSCs), named for their potential to give rise to tumors, are tissue specific and can migrate, properties that provide support to the concept of these cells being the underlying basis of field cancerization. In the oral mucosa, wherein the differentiated epithelial cells have a high renewal rate (every 14 days) [145], the long-time residents of epithelium, the slowly dividing, oral stem cells (SCs), are more likely to accumulate the necessary hits mandatory for transformation. The detection of CSC markers in the tumor-adjacent normal mucosa provides evidence toward their role in cancerization. In OSCC, studies have revealed that tumor-adjacent normal tissues of recurrent and the non-recurrent patients showed expression of CSC markers such as ATR, CD44, ABCG1, and ANKRD50 [146, 147]. Studies in rat oral carcinogenesis models have shown an expression of SC-related markers such as Oct4 and Sox2 in the normal and transforming oral mucosa. These results were further validated when these markers were expressed in the non-cancer tissue adjacent to the tumor and in the precancerous lesions of oral cancer patients [148].

Similar evidences are available in other tumors also; single and multiple clonal tumors with CSC markers have been reported in the gastrointestinal tract. It has been reported that normal human gastric stem cells can acquire mutations, proliferate, and ultimately lead to the formation of a new patch of abnormal cells in the preneoplastic field [149, 150]. Injury to lung tissue is also reported to lead to a deregulated repair of stem cells, which then form a clonal group of indefinitely self-renewing daughter cells in the normal mucosa. Additional mutations lead to proliferation and finally result in a stepwise progression of the disease in the tissue [151]. Studies in breast cancer samples also provided a clinical correlation; CD44+/CD24+ cells were enriched in the adjacent mucosa of patients with triple-negative breast cancers indicating a possible prognostic significance [152, 153]. A recent review from our lab has comprehensively cataloged the possible implications of CSCs in field cancerization. A multitude of CSC-markers have been associated with the various processes involved in field cancerization (Table 1.3).

Table 1.3 CSC-related markers that could aid in detection of field cancerization [154]

Types of marker	Marker	Cancer stem cell relation	Role in field cancerization	Detection in adjacent mucosa
Pluripotent markers	Oct4	Cancer stem cell marker in oral cancer; associated with prognosis	Dedifferentiation of tumor/mature cells	✔
	Sox2	SOX2 has role in regulating cancer stem cell properties of pancreatic cancer cells	Dedifferentiation of tumor/mature cells; tumor Initiation	✔
	Nanog	Moon et al. have reported that Nanog has a role in genesis of cancer stem cells in GBM	Dedifferentiation of tumor/mature cells	✘
Aldehyde dehydrogenase	ALDH1A1	ALDH1+/CD44+ cells show increased migration and tumor initiation	*Intraepithelial migration, tumor initiation*	✘
Drug transporter	ABCG2	Stem cell marker imparting drug resistance in HNSCC; ABCG2+ cells increased tumor initiation	*Tumor initiation/ drug resistance*	✘
Adhesion molecule	CD44	CSC marker in HNSCC	*Tumor initiation*	✔
	CD133	Putative CSC marker in brain, prostrate, and head and neck cancer	*Tumor initiation*	✘

(continued)

Table 1.3 continued

Types of marker	Marker	Cancer stem cell relation	Role in field cancerization	Detection in adjacent mucosa
EMT markers	E-cadherin	Marker of EMT and CSCs (breast cancer spheroids positive for E-cadherin)	*Epithelial migration*	✔
	S100A4	Putative CSC marker in HNSCC	*Epithelial migration*	✔
	MMPs	Implicated in the invasive behavior of CSCs in colorectal cancer and OSCC	*Epithelial migration*	✔
	SNAIL	EMT marker that maintains self-renewal properties of CSCs	*Tumor initiation/ migration*	✔
	S100A8	Progression of disease in colorectal carcinoma and migration of cancer stem cells	*Epithelial migration*	✔
Tumor supressor genes/ oncogenes/ cell cycle regulatory gene	Cyclin D1	Induces EMT in CSCs in ovarian cancer	*Epithelial migration*	✔
	K-Ras	Mutations in K-Ras activate CSCs contributing toward tumorigenesis as well as metastasis in the cells	*Tumor initiation*	✔
Differentiation antigen	CK8/18, CK19	CK8/18 is expressed CSCs of papillary carcinoma; CK19 in cutaneous epithelial lesions	*Proliferation/ initiation*	✔
	Telomerase	Telomerase enzymatic blockers, such as Imetelstat, have been shown to decrease CSC populations	*Tumorigenesis*	✔
	RAR	Expression correlates with CSC expression in pancreatic cancer	*Tumorigenesis*	✔
Proliferation marker	Ki67	Ki67 is a marker of cancer stem cell of glioblastoma	*Proliferation*	✔
Growth factors/ receptors	EGFR	EGFR is highly expressed in CD133 positive glioblastoma	*Tumorigenesis*	✔
	VEGF	EMT-induced VEGF-A expression can lead to tumorigenesis	*Angiogenesis/ tumor initiation*	✔
Drug-resistant genes	ATR	Inhibition of ATR abrogates tumorigenicity of colon cancer cells through depletion of CD133 positive cancer stem cell population	*Drug resistance*	✔

Assessment of the role of the cancer stem cells in field cancerization process thus gives rise to a new concept that can further be employed toward identification of novel markers. The concept, if established, can enable identification of predictors of neoplastic transformation in the histologically normal, tumor-adjacent mucosa that can be evaluated for clinical utility.

1.2.4.1 Implication of Field Cancerization in Diagnosis and Therapy in Oral Cancer

In the current scenario, there is a lack of prognostic biomarkers that can predict recurrence and formation of second primary tumor in HNSCC. The extensive marker repertoire that was identified down the decades has neither been used clinically in the assessment of surgical margins nor toward accurate prediction of disease recurrence in HNSCC patients. The most effective way of confronting the disease relapse is accurate prediction of transforming clonal events in surrounding normal epithelium at the time of cancer resection. Knowledge of early events of carcinogenesis can be used to identify residual clonal populations in tumor margins by molecular analysis to more accurately assess the successful surgical resection.

The concept of field cancerization implies cause-effect reasoning for the generation of secondary and recurrent tumors. Future research focus should be on identification of molecules and molecular events that affect prognosis. New tumor markers have yet to be clinically applied with the ultimate goals including the prevention and effective treatment of head and neck cancer. Delineating the role of cancer stem cells in field cancerization would provide a different approach toward understanding of their role in the progression of the disease, besides providing cues for developing novel treatment modalities targeting these cancer stem cells. By using these tumor-initiating cell signatures as biomarkers on progenitor cells, postsurgical state of patients can be known, which can customize the therapeutic regime and improve the efficacy of current cancer therapies.

1.2.4.2 Definition of Terminology

Loss of Heterozygosity and Single Nucleotide Polymorphism

Most diploid human somatic cells contain two copies of the genome, one from each parent (chromosome pair). Each copy contains approximately 3 billion bases (adenine (A), guanine (G), cytosine (C), or thymine (T)). For the majority of positions in the genome, the base present is consistent between individuals; however, a small percentage may contain different bases (usually one of two, for instance, “A” or “G”), and these positions are called “single nucleotide polymorphisms” or “SNPs.” When the genomic copies derived from each parent have different bases for these polymorphic regions (SNPs), the region is said to be heterozygous. Most of the chromosomes within somatic cells of individuals are paired, allowing for SNP locations to be potentially heterozygous. However, one parental copy of a region can sometimes be lost, which results in the region having just one copy. The single copy cannot be heterozygous at SNP locations, and therefore the region shows loss of heterozygosity (LOH). Loss of heterozygosity due to loss of one parental copy in a region is also called hemizygosity in that region.

Microsatellites It is also known as *simple sequence repeats* (SSRs) or *short tandem repeats* (STRs). They are repeating sequences of 2–5 base pairs of DNA. They are unique to an individual or a tumor. It can be used as molecular markers in STR analysis, for family, population, and tumor clonality. They can also be used for studies of gene duplication or deletion, marker-assisted selection, and fingerprinting.

References

1. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med. 2003;14(1):47–62.
2. Feng J-Q, et al. Expression of cancer stem cell markers ALDH1 and Bmi1 in oral erythroplakia and the risk of oral cancer. J Oral Pathol Med. 2013;42(2):148–53.
3. Axell T, et al. Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18-21 1994. International Collaborative Group on Oral White Lesions. J Oral Pathol Med. 1996; 25(2):49–54.
4. Reichart PA, Philipsen HP. Oral erythroplakia – a review. Oral Oncol. 2005;41(6):551–61.
5. Silverman Jr S, Gorsky M. Proliferative verrucous leukoplakia: a follow-up study of 54 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997;84(2):154–7.
6. Zakrzewska JM, et al. Proliferative verrucous leukoplakia: a report of ten cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1996;82(4):396–401.
7. Lai DR, et al. Clinical evaluation of different treatment methods for oral submucous fibrosis. A 10-year experience with 150 cases. J Oral Pathol Med. 1995;24(9):402–6.
8. Lian Ie B, Tseng YT, Su CC, Tsai KY. Progression of precancerous lesions to oral cancer: results based on the Taiwan National Health Insurance Database. Oral Oncol. 2013; 49(5):427–30.
9. Scully C, et al. Update on oral lichen planus: etiopathogenesis and management. Crit Rev Oral Biol Med. 1998;9(1):86–122.
10. Silverman Jr S, Bahl S. Oral lichen planus update: clinical characteristics, treatment responses, and malignant transformation. Am J Dent. 1997;10(6):259–63.
11. Speight PM. Update on oral epithelial dysplasia and progression to cancer. Head Neck Pathol. 2007;1(1):61–6.
12. Jagannathan N, Ramani P, Premkumar P, Natesan A, Sherlin HJ. Epithelial maturation pattern of dysplastic epithelium and normal oral epithelium exposed to tobacco and alcohol: a scanning electron microscopic study. Ultrastruct Pathol. 2013;37(3):171–5.
13. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, Corio R, Lee D, Greenberg B, Koch W, Sidransky D. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res. 1996;56(11):2488–92.
14. Braakhuis BJ, Leemans CR, Brakenhoff RH. A genetic progression model of oral cancer: current evidence and clinical implications. J Oral Pathol Med. 2004;33(6):317–22.
15. Partridge M, Emilion G, Pateromichelakis S, A'Hern R, Phillips E, Langdon J. Allelic imbalance at chromosomal loci implicated in the pathogenesis of oral precancer, cumulative loss and its relationship with progression to cancer. Oral Oncol. 1998;34(2):77–83.
16. Zhou X, Jordan RC, Li Y, Huang BL, Wong DT. Frequent allelic imbalances at 8p and 11q22 in oral and oropharyngeal epithelial dysplastic lesions. Cancer Genet Cytogenet. 2005;161(1):86–9.
17. Zhang L, Cheng X, Li Y, Poh C, Zeng T, Priddy R, Lovas J, Freedman P, Daley T, Rosin MP. High frequency of allelic loss in dysplastic lichenoid lesions. Lab Invest. 2000; 80(2):233–7.

18. Rosin MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J, Berean K, Epstein JB, Priddy R, Le ND, Zhang L. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin Cancer Res. 2000;6(2):357–62.
19. Chang SS, Califano J. Current status of biomarkers in head and neck cancer. J Surg Oncol. 2008;97(8):640–3.
20. Graveland AP, Bremmer JF, de Maaker M, Brink A, Cobussen P, Zwart M, Braakhuis BJ, Bloemena E, van der Waal I, Leemans CR, Brakenhoff RH. Molecular screening of oral precancer. Oral Oncol. 2013;49(12):1129–35.
21. Tabor MP, Braakhuis BJ, van der Wal JE, van Diest PJ, Leemans CR, Brakenhoff RH, Kummer JA. Comparative molecular and histological grading of epithelial dysplasia of the oral cavity and the oropharynx. J Pathol. 2003;199(3):354–60.
22. Soni S, et al. Alterations of rb pathway components are frequent events in patients with oral epithelial dysplasia and predict clinical outcome in patients with squamous cell carcinoma. Oncology. 2005;68(4-6):314–25.
23. Schoelch ML, Le QT, Silverman Jr S, McMillan A, Dekker NP, Fu KK, Ziober BL, Regezi JA. Apoptosis-associated proteins and the development of oral squamous cell carcinoma. Oral Oncol. 1999;35(1):77–85.
24. Brennan PA, Conroy B, Spedding AV. Expression of inducible nitric oxide synthase and p53 in oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90(5):624–9.
25. Cruz IB, et al. p53 expression above the basal cell layer in oral mucosa is an early event of malignant transformation and has predictive value for developing oral squamous cell carcinoma. J Pathol. 1998;184(4):360–8.
26. Shintani S, et al. Expression of cell cycle control proteins in normal epithelium, premalignant and malignant lesions of oral cavity. Oral Oncol. 2002;38(3):235–43.
27. Jordan RC, Bradley G, Slingerland J. Reduced levels of the cell-cycle inhibitor p27Kip1 in epithelial dysplasia and carcinoma of the oral cavity. Am J Pathol. 1998;152(2):585–90.
28. Schoelch ML, et al. Cell cycle proteins and the development of oral squamous cell carcinoma. Oral Oncol. 1999;35(3):333–42.
29. Gologan O, Barnes EL, Hunt JL. Potential diagnostic use of p16INK4A, a new marker that correlates with dysplasia in oral squamoproliferative lesions. Am J Surg Pathol. 2005; 29(6):792–6.
30. Soria JC, et al. Telomerase activation cooperates with inactivation of p16 in early head and neck tumorigenesis. Br J Cancer. 2001;84(4):504–11.
31. Scambia G, Lovergine S, Masciullo V. RB family members as predictive and prognostic factors in human cancer. Oncogene. 2006;25(38):5302–8.
32. Tanaka N, et al. pRb2/p130 protein expression is correlated with clinicopathologic findings in patients with oral squamous cell carcinoma. Cancer. 2001;92(8):2117–25.
33. Tanaka N, et al. Expression of Rb, pRb2/p130, p53, and p16 proteins in malignant melanoma of oral mucosa. Oral Oncol. 2001;37(3):308–14.
34. Lee JI, Soria JC, Hassan KA, El-Naggar AK, Tang X, Liu DD, Hong WK, Mao L. Loss of PTEN expression as a prognostic marker for tongue cancer. Arch Otolaryngol Head Neck Surg. 2001;127(12):1441–5.
35. Torres-Rendon A, et al. Expression of Mcm2, geminin and Ki67 in normal oral mucosa, oral epithelial dysplasias and their corresponding squamous-cell carcinomas. Br J Cancer. 2009;100(7):1128–34.
36. Feng CJ, et al. Expression of Mcm7 and Cdc6 in oral squamous cell carcinoma and precancerous lesions. Anticancer Res. 2008;28(6A):3763–9.
37. Gonzalez-Moles MA, et al. Suprabasal expression of Ki-67 antigen as a marker for the presence and severity of oral epithelial dysplasia. Head Neck. 2000;22(7):658–61.
38. Iamaroon A, et al. Co-expression of p53 and Ki67 and lack of EBV expression in oral squamous cell carcinoma. J Oral Pathol Med. 2004;33(1):30–6.
39. Kodani I, et al. Expression of minichromosome maintenance 2 (MCM2), Ki-67, and cell-cycle-related molecules, and apoptosis in the normal-dysplasia-carcinoma sequence of the oral mucosa. Pathobiology. 2001;69(3):150–8.

40. Abbas NF, et al. Immunohistochemical study of p53 and angiogenesis in benign and preneoplastic oral lesions and oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103(3):385–90.
41. Pazouki S, et al. The association between tumour progression and vascularity in the oral mucosa. J Pathol. 1997;183(1):39–43.
42. Gandolfo M, et al. Increased subepithelial vascularization and VEGF expression reveal potentially malignant changes in human oral mucosa lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;111(4):486–93.
43. Ravi D, et al. Angiogenesis during tumor progression in the oral cavity is related to reduced apoptosis and high tumor cell proliferation. Oral Oncol. 1998;34(6):543–8.
44. Shivamallappa SM, et al. Role of angiogenesis in oral squamous cell carcinoma development and metastasis: an immunohistochemical study. Int J Oral Sci. 2011;3(4):216–24.
45. Li C, et al. Microvessel density and expression of vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived endothelial growth factor in oral squamous cell carcinomas. Int J Oral Maxillofac Surg. 2005;34(5):559–65.
46. Kushner J, Bradley G, Jordan RC. Patterns of p53 and Ki-67 protein expression in epithelial dysplasia from the floor of the mouth. J Pathol. 1997;183(4):418–23.
47. Xiaolian G, et al. p63 contributes to cell invasion and migration in squamous cell carcinoma of the head and neck. Cancer Lett. 2008;263.
48. Das RK, Pal M, Barui A, Paul RR, Chakraborty C, Ray AK, Sengupta S, Chatterjee J. Assessment of malignant potential of oral submucous fibrosis through evaluation of p63, E-cadherin and CD105 expression. J Clin Pathol. 2010;63(10):894–9.
49. Wang Y, et al. Akt/Ezrin Tyr353/NF-kappaB pathway regulates EGF-induced EMT and metastasis in tongue squamous cell carcinoma. Br J Cancer. 2014;110(3):695–705.
50. Villaret D, et al. Identification of genes overexpressed in head and neck squamous cell carcinoma using a combination of complementary DNA subtraction and microarray analysis. Laryngoscope. 2000;110(3 Pt 1):374–81.
51. Kannan S, et al. Differential expression of cytokeratin proteins during tumour progression in oral mucosa. Epithelial Cell Biol. 1994;3(2):61–9.
52. Kannan S, et al. Alterations in expression of terminal differentiation markers of keratinocytes during oral carcinogenesis. Pathobiology. 1994;62(3):127–33.
53. Cervigne NK, Reis PP, Machado J, Sadikovic B, Bradley G, Galloni NN, Pintilie M, Jurisica I, Perez-Ordonez B, Gilbert R, Gullane P, Irish J, Kamel-Reid S. Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. Hum Mol Genet. 2009;18(24):4818–29.
54. Yang CC, Hung PS, Wang PW, Liu CJ, Chu TH, Cheng HW, Lin SC. miR-181 as a putative biomarker for lymph-node metastasis of oral squamous cell carcinoma. J Oral Pathol Med. 2011;40(5):397–404.
55. Hui AB, Lenarduzzi M, Krushel T, Waldron L, Pintilie M, Shi W, Perez-Ordonez B, Jurisica I, O'Sullivan B, Waldron J, Gullane P, Cummings B, Liu FF. Comprehensive MicroRNA profiling for head and neck squamous cell carcinomas. Clin Cancer Res. 2010;16(4):1129–39.
56. Abrahao AC, Bonelli BV, Nunes FD, Dias EP, Cabral MG. Immunohistochemical expression of p53, p16 and hTERT in oral squamous cell carcinoma and potentially malignant disorders. Braz Oral Res. 2011;25(1):34–41.
57. Clague J, et al. Genetic variation in MicroRNA genes and risk of oral premalignant lesions. Mol Carcinog. 2010;49(2):183–9.
58. Chen HH, et al. Expression of human telomerase reverse transcriptase (hTERT) protein is significantly associated with the progression, recurrence and prognosis of oral squamous cell carcinoma in Taiwan. Oral Oncol. 2007;43(2):122–9.
59. Zhang L, Zhang W. Telomerase hTR and hTRT gene expression in oral precancerous lesions and squamous cell carcinomas. Chin J Dent Res. 1999;2(2):43–8.
60. Kim HR, et al. Elevated expression of hTERT is associated with dysplastic cell transformation during human oral carcinogenesis in situ. Clin Cancer Res. 2001;7(10):3079–86.

61. Liao J, et al. Telomerase activity in oral and maxillofacial tumors. Oral Oncol. 2000; 36(4):347–52.
62. Pannone G, et al. Prognostic value of human telomerase reverse transcriptase gene expression in oral carcinogenesis. Int J Oncol. 2007;30(6):1349–57.
63. Routray S, Kheur SM, Kheur M. Osteopontin: a marker for invasive oral squamous cell carcinoma but not for potentially malignant epithelial dysplasias. Ann Diagn Pathol. 2013. pii: S1092-9134(13)00034-8. doi:10.1016/j.anndiagpath.2013.03.005. [Epub ahead of print].
64. Pontes HA, Pontes FS, Fonseca FP, de Carvalho PL, Pereira EM, de Abreu MC, de Freitas Silva BS, dos Santos Pinto Jr D. Nuclear factor kappaB and cyclooxygenase-2 immunoexpression in oral dysplasia and oral squamous cell carcinoma. Ann Diagn Pathol. 2013;17(1):45–50.
65. Santhi WS, et al. NF-kappaB and COX-2 during oral tumorigenesis and in assessment of minimal residual disease in surgical margins. Exp Mol Pathol. 2006;81(2):123–30.
66. Yarden Y, Schlessinger J. Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor. Biochemistry. 1987;26(5):1443–51.
67. Nishikawa R, et al. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad Sci. 1994;91.
68. Taoudi Benchekroun M, et al. Epidermal growth factor receptor expression and gene copy number in the risk of oral cancer. Cancer Prev Res (Phila). 2010;3(7):800–9.
69. Lourenco SV, Coutinho-Camillo CM, Buim ME, Pereira CM, Carvalho AL, Kowalski LP, Soares FA. Oral squamous cell carcinoma: status of tight junction claudins in the different histopathological patterns and relationship with clinical parameters. A tissue-microarray-based study of 136 cases. J Clin Pathol. 2010;63(7):609–14.
70. Ries J, et al. Evaluation of MAGE-A expression and grade of dysplasia for predicting malignant progression of oral leukoplakia. Int J Oncol. 2012;41(3):1085–93.
71. Nayyar AS, et al. Serum total protein, albumin and advanced oxidation protein products (AOPP)--implications in oral squamous cell carcinoma. Malays J Pathol. 2012;34(1):47–52.
72. Arora S, et al. Stromelysin 3, Ets-1, and vascular endothelial growth factor expression in oral precancerous and cancerous lesions: correlation with microvessel density, progression, and prognosis. Clin Cancer Res. 2005;11(6):2272–84.
73. Sudha VM, Hemavathy S. Role of bcl-2 oncoprotein in oral potentially malignant disorders and squamous cell carcinoma: an immunohistochemical study. Indian J Dent Res. 2011;22(4):520–5.
74. Loro LL, Johannessen AC, Vintermyr OK. Decreased expression of bcl-2 in moderate and severe oral epithelia dysplasias. Oral Oncol. 2002;38(7):691–8.
75. de Vicente JC, et al. Podoplanin expression in oral leukoplakia: tumorigenic role. Oral Oncol. 2013;49(6):598–603.
76. Tripathi SC, et al. Nuclear S100A7 is associated with poor prognosis in head and neck cancer. PLoS One. 2010;5(8), e11939.
77. Dalerba P, Cho R, Clarke M. Cancer stem cells: models and concepts. Annu Rev Med. 2007;58:267–84.
78. Prince M, Ailles L. Cancer stem cells in head and neck squamous cell cancer. J Clin Oncol. 2008;26(17):2871–5.
79. Bhaijee F, et al. Cancer stem cells in head and neck squamous cell carcinoma: a review of current knowledge and future applications. Head Neck. 2012;34(6):894–9.
80. Luo W, et al. Embryonic stem cells markers SOX2, OCT4 and Nanog expression and their correlations with epithelial-mesenchymal transition in nasopharyngeal carcinoma. PLoS One. 2013;8(2), e56324.
81. Kim J, et al. Oct4-induced pluripotency in adult neural stem cells. Cell. 2009;136(3):411–9.
82. Tai M-H, et al. Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis. 2005;26(2):495–502.
83. Hochedlinger K, et al. Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. Cell. 2005;121(3):465–77.
84. Vigneswaran N, Beckers S, Waigel S, Mensah J, Wu J, Mo J, Fleisher KE, Bouquot J, Sacks PG, Zacharias W. Increased EMMPRIN (CD 147) expression during oral carcinogenesis. Exp Mol Pathol. 2006;80(2):147–59.

85. Chen YC, Chen YW, Hsu HS, Tseng LM, Huang PI, Lu KH, Chen DT, Tai LK, Yung MC, Chang SC, Ku HH, Chiou SH, Lo WL. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. Biochem Biophys Res Commun. 2009;385(3):307–13.
86. Abdulmajeed A, Dalley A, Farah C. Putative cancer stem cell marker expression in oral epithelial dysplasia and squamous cell carcinoma. J Oral Pathol Med. 2013; 42(10):755–60.
87. Liu W, Wu L, Shen XM, Shi LJ, Zhang CP, Xu LQ, Zhou ZT. Expression patterns of cancer stem cell markers ALDH1 and CD133 correlate with a high risk of malignant transformation of oral leukoplakia. Int J Cancer. 2013;132(4):868–74.
88. Ishiwata T, Matsuda Y, Naito Z. Nestin in gastrointestinal and other cancers: effects on cells and tumor angiogenesis. World J Gastroenterol. 2011;17(4):409–18.
89. Lim Y, et al. Cancer stem cell traits in squamospheres derived from primary head and neck squamous cell carcinomas. Oral Oncol. 2011;47(2):83–91.
90. Günthert U, et al. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell. 1991;65(1):13–24.
91. Kamarajan P, Shin JM, Qian X, Matte B, Zhu JY, Kapila YL. ADAM17-mediated CD44 cleavage promotes orasphere formation or stemness and tumorigenesis in HNSCC. Cancer Med. 2013;2(6):793–802.
92. Ravindran G, Devaraj H. Aberrant expression of CD133 and musashi-1 in preneoplastic and neoplastic human oral squamous epithelium and their correlation with clinicopathological factors. Head Neck. 2011;34(8):1129–35.
93. Baeten J, et al. Molecular imaging of oral premalignant and malignant lesions using fluorescently labeled lectins. Transl Oncol. 2014;7(2):213–20.
94. Al-Tarawneh SK, et al. Defining salivary biomarkers using mass spectrometry-based proteomics: a systematic review. OMICS. 2011;15(6):353–61.
95. Brinkmann O, et al. Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population. Oral Oncol. 2011;47(1):51–5.
96. Hu S, et al. Salivary proteomics for oral cancer biomarker discovery. Clin Cancer Res. 2008;14(19):6246–52.
97. Fountzilas G. Retinoids in the management of head and neck cancer. An update. J Chemother. 1994;6(2):127–38.
98. Gorsky M, Epstein JB. The effect of retinoids on premalignant oral lesions: focus on topical therapy. Cancer. 2002;95(6):1258–64.
99. Cheng AL, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res. 2001;21(4B):2895–900.
100. Grandhi BK, et al. A novel combinatorial nanotechnology-based oral chemopreventive regimen demonstrates significant suppression of pancreatic cancer neoplastic lesions. Cancer Prev Res (Phila). 2013;6(10):1015–25.
101. Johnson JJ, Mukhtar H. Curcumin for chemoprevention of colon cancer. Cancer Lett. 2007;255(2):170–81.
102. Bianchini C, et al. Targeted therapy in head and neck cancer. Tumori. 2011;97(2):137–41.
103. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer. 2011;11(1):9–22.
104. Braakhuis BJ, et al. Second primary tumors and field cancerization in oral and oropharyngeal cancer: molecular techniques provide new insights and definitions. Head Neck. 2002; 24(2):198–206.
105. Feller LL, et al. Oral squamous cell carcinoma in relation to field precancerisation: pathobiology. Cancer Cell Int. 2013;13(1):31.
106. van Oijen MG, Slootweg PJ. Oral field cancerization: carcinogen-induced independent events or micrometastatic deposits? Cancer Epidemiol Biomarkers Prev. 2000;9(3):249–56.
107. Simon R, et al. Cytogenetic analysis of multifocal bladder cancer supports a monoclonal origin and intraepithelial spread of tumor cells. Cancer Res. 2001;61(1):355–62.