Head and Neck Cancer Clinics
Series Editors: Rehan Kazi · Raghav C. Dwivedi

Peter A. Brennan
Tom Aldridge
Raghav C. Dwivedi *Editors*

Premalignant Conditions of the Oral Cavity

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Head and Neck Cancer Clinics

Series editors

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Head and Neck Cancer (HNC) is a major challenge to public health. Its management involves a multidisciplinary team approach, which varies depending on the subtle differences in the location of the tumour, stage and biology of disease and availability of resources. In the wake of rapidly evolving diagnostic technologies and management techniques, and advances in basic sciences related to HNC, it is important for both clinicians and basic scientists to be up-to-date in their knowledge of new diagnostic and management protocols. This series aims to cover the entire range of HNC-related issues through independent volumes on specific topics. Each volume focuses on a single topic relevant to the current practice of HNC, and contains comprehensive chapters written by experts in the field. The reviews in each volume provide vast information on key clinical advances and novel approaches to enable a better understanding of relevant aspects of HNC. Individual volumes present different perspectives and have the potential to serve as stand-alone reference guides. We believe these volumes will prove useful to the practice of head and neck surgery and oncology, and medical students, residents, clinicians and general practitioners seeking to develop their knowledge of HNC will benefit from them.

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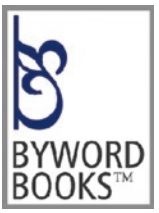
Peter A. Brennan • Tom Aldridge
Raghav C. Dwivedi
Editors

Premalignant Conditions of the Oral Cavity



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Preface

Oral cancer is a global healthcare problem with an increasing incidence year on year. While there have been many advances in the diagnosis, staging, treatment and reconstruction and rehabilitation following ablative surgery, the crude 5-year survival rates still remain at approximately 50%. Systemic chemotherapy using some of the newer monoclonal antibodies as well as the prompt treatment of early stage disease are associated with increased survival. New advances in surgery and radiotherapy including for example intensity-modulated radiotherapy (IMRT) are reducing post-treatment complications.

Oral squamous cell carcinoma (OSCC) is often related to smoking, alcohol consumption and other habits including betel or areca nut chewing. p16 has been more recently implicated in the aetiology of tumours of the oropharynx including tonsil and tongue base. Some OSCCs seem to arise de novo in clinically normal looking mucosa, while others occur following a premalignant disease. Therefore, the early recognition, diagnosis and management of these pre-cancerous diseases are crucial to improve survival and reduce morbidity for patients.

Research in both pre-malignant diseases and OSCC continues at a rapid pace, and it can be difficult to keep abreast of all developments particularly with some of the new and exciting molecular pathways and understanding of pathogenesis. In this unique new book, we have brought together respected experts and colleagues from around the world to provide a contemporary overview of the common premalignant conditions affecting the oral cavity. Following an overview which includes information on epidemiology and diagnosis, we have focused on the common diseases leading to potential malignant change in the oral cavity and their management. We have included cutting-edge research and developments across the specialties of oral medicine, oral pathology and OMFS.

With such a vast and ever-increasing subject, we apologise in advance for any omissions and would be grateful to receive feedback from readers with suggestions for the next edition of this book.

Portsmouth, UK — Peter A. Brennan
Portsmouth, UK — Tom Aldridge
Glasgow, UK — Raghav C. Dwivedi

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About the Editors

Peter A. Brennan, MD, FRCS, FRCSI, Hon FRCS Professor Peter Brennan is a Consultant Oral and Maxillofacial Surgeon at the Queen Alexandra Hospital, Portsmouth, UK, with an interest in head and neck oncology and reconstruction. He has a personal chair in surgery in recognition of his research and education profile, publishing over 530 papers to date as well as editing five major textbooks (including lead editor of the two-volume *Maxillofacial Surgery*) used successfully worldwide. He is lead editor for the new *Gray's Surgical Anatomy*—sister to the famous *Gray's Anatomy* itself.

Peter is committed to teaching and education at all levels and was previous Honorary Editor of the *British Journal of Oral and Maxillofacial Surgery*. In addition to reviewing for many reputed journals, he is the current editor of the *Journal of Oral Pathology and Medicine*—one of the most well-respected journals in this specialty area. Peter has research interests in oral cancer, neck anatomy, patient safety and human factors.

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Raghav Dwivedi, FRCS (Glas), PhD, FRCS (ORL-HNS) Raghav Dwivedi is a Consultant Head Neck Surgeon at the Queen Elizabeth University Hospital, Glasgow. He completed ENT and head neck surgery specialty training from the UK and India. He also completed head neck research fellowship at the Royal Marsden Hospital and the Institute of Cancer Research, London, and head neck surgical fellowships at Nottingham University Hospital, Cambridge University Hospitals, Portsmouth University Hospitals, and Imperial College, London. He holds an intercollegiate FRCS (ORL-HNS) from the Royal College of Surgeons of England; PhD from the Institute of Cancer Research, University of London, UK; and MS (ENT) from the King George's Medical University, India.

He is a dedicated head neck and ENT surgeon and has a unique blend of high-quality clinical and research experience. His areas of interest are minimally invasive head neck, thyroid and parathyroid surgery, HPV-related head neck cancers and outcome research. To date he has published 80 scientific papers in peer-reviewed indexed journals, 21 chapters (including one in the upcoming edition of Scott-Brown's *Otolaryngology: Head and Neck Surgery*) and edited one head neck surgery book. He has also served on the editorial board of six specialty journals and has been scientific reviewer for 35 peer-reviewed indexed journals including *BMJ*, *Cancer*, *Head and Neck*, *Oral Oncology* and *Surgical Oncology*.

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Chapter 1
Introduction

Peter A. Brennan and Tom Aldridge

We have invited leading experts from around the world to contribute to this book on the management of oral premalignancy. The book includes an up-to-date and comprehensive analysis of risk factors and systemic conditions that can lead to oral squamous cell carcinoma (OSCC) as well as a description of carcinogenesis at both molecular and genetic levels. Specific premalignant conditions are discussed, and detailed management strategies are provided. In the remaining chapters, current, interesting and useful information on the various premalignant conditions are included which we hope will enhance clinical practice and patient care.

In this introduction, we provide a brief overview of the epidemiology of oral premalignant disease and the potential impact that it has on our patients. We also give an overview on the structural and mucosal anatomy of the oral cavity and lips that makes this area such a challenging and complex location to manage.

Oral Premalignancy

Oral cavity cancer accounts for approximately 3% of all cancers. Most are oral squamous cell carcinoma (OSCC), and disappointingly the 5-year survival has not significantly improved over the last few decades, despite many advances in diagnosis, imaging and treatment modalities. Quality of life following oral cancer treatment has also improved with advances in free tissue transfer and targeted therapy including intensity-modulated radiotherapy (IMRT) which can spare adjacent structures such as the salivary glands and cervical spinal cord. Many OSCC tumours develop from premalignant conditions of the oral mucosa which are sometimes not detected or diagnosed before the cancer itself. Premalignant conditions have huge

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geographical, socioeconomic and population variation with an accepted prevalence of 1–5% and are most commonly found in the buccal mucosa, lower gingivae, tongue and floor of the mouth [1].

The World Health Organization originally recommended the terms 'precancerous lesions' and 'precancerous conditions'. A precancerous lesion is a morphologically altered tissue in which oral cancer is more likely to occur than in apparently normal counterpart. A precancerous condition is a generalised state associated with significantly increased risk of cancer. However, in 2005 these terms were simplified to 'potentially malignant disorders' to eliminate confusion from the previous used terminology, definitions and classifications of oral lesions with a predisposition to malignant transformation (Fig. 1.1) [2].

Oral precancerous lesions take many forms with leukoplakia, oral submucous fibrosis (OSMF) and oral erythroplakia being the most common (Fig. 1.2). There are other presentations of systemic conditions that can also be premalignant, such as xeroderma pigmentosum and Fanconi's anaemia. The link between carcinogenesis and immunodeficiency is also well known [3].

Although our knowledge is improving, the aetiology of premalignant conditions of oral mucosa is still incompletely understood [4]. There are well-recognised risk factors such as tobacco chewing, tobacco smoking, areca nut (for OSMF) and alcohol. While tobacco chewing is a major risk factor for oral leukoplakia, OSMF and erythroplakia, tobacco smoking may be a risk factor for oral leukoplakia. Alcohol drinking may increase the risk by 1.5-fold for oral leukoplakia, by twofold for OSMF, and threefold for erythroplakia.

The risk of malignant change in the external lip can occur with use of the above agents, but actinic damage following chronic sun exposure (UVA light) is the major risk factor associated with lower lip SCC (Fig. 1.3). The lower lip is at particular risk due to its reduced keratinised mucosa, reduced melanocyte number and orientation perpendicular to the sun and lack of protection from all but the widest brimmed hats.

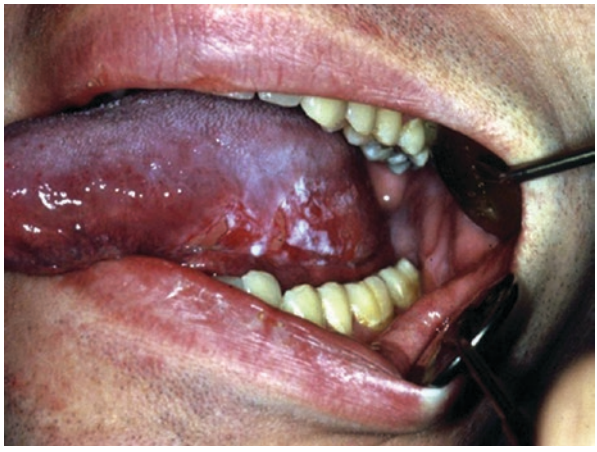

Fig. 1.1 Leukoplakia, left side of the tongue

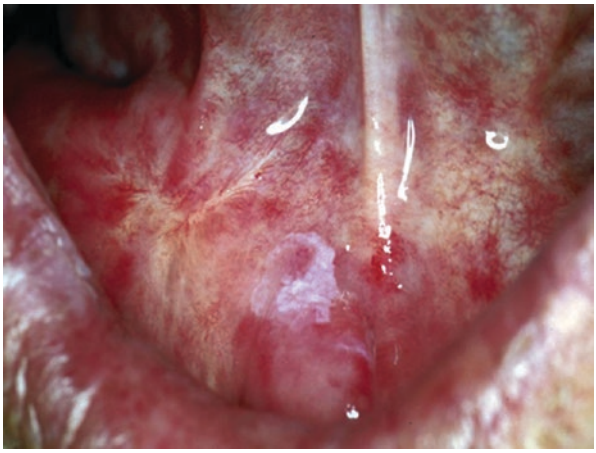

Fig. 1.2 Leukoplakia, floor of the mouth

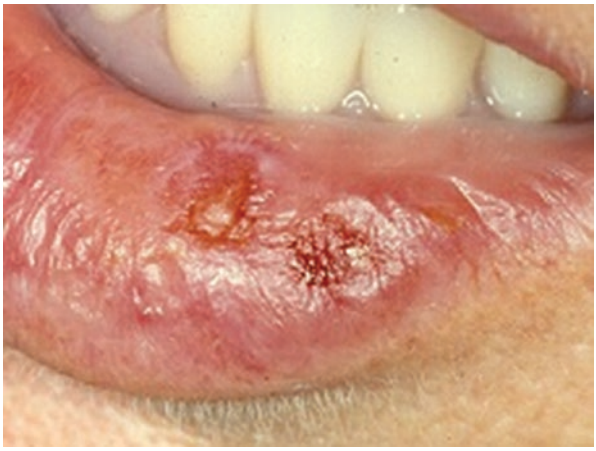

Fig. 1.3 Actinic keratosis, lower lip

For some strange reason, the minor salivary gland cancers well known in the upper lip are rarely seen in the lower lip, and almost all cancers are SCC from chronic sun exposure or tobacco use.

Embryology

The oral cavity develops from an ectoderm lined depression called the stomodeum. It is initially separated from the endoderm lined foregut by the transient buccopharyngeal membrane. Between the fourth and eighth week in utero, the frontal

prominence, together with the maxilla and mandible swellings of the first pharyngeal arch, develops to deepen the stomodeum. The pharyngeal arches each with their unique combinations of a nerve, muscle and cartilage go on to form the face and neck.

The first pharyngeal arch mesenchyme forms the maxilla which undergoes intramembranous ossification, and the mandible develops from intramembranous ossification of Meckel's cartilage. The muscles of mastication form from the first arch and hence receive motor innervation from the trigeminal nerve (fifth cranial nerve). The tongue develops concurrently with fusing of tissue from two lingual swellings and the tuberculum impar all derived from the first pharyngeal arch. These swellings form the anterior two thirds of the tongue and fuse with swelling from the second, third and fourth pharyngeal arches which themselves form the posterior one third. This explains the innervation of the posterior third innervation from the glossopharyngeal nerve.

Oral Mucosa

The oral cavity contains a complex variety of tissues from the hardest enamel to delicate salivary gland parenchyma. The oral cavity fuses with the skin at the vermillion and with the pharyngeal mucosa at the soft palate. The functions of the oral cavity are varied and require durability, special senses, protection and regeneration.

The oral mucosa itself consists of two layers with a surface stratified squamous epithelium and a deeper lamina propria. The histology of these components varies depending on the location. The epithelium is further divided into:

- Stratum basale
- Stratum spinosum
- Stratum granulosum
- Stratum corneum

The degree of keratinisation varies between location and function with keratinised mucosa being found on the attached gingivae, hard palate and dorsum of the tongue. Non-keratinised mucosa is found on the soft palate, inner lips, cheek, floor of the mouth and ventral tongue. These surfaces can become keratinised after periods of friction, for example, from poorly fitting denture or cheek biting (linea alba) or chemical irritation such as in 'smoker's palate' (nicotinic stomatitis). The classic sublingual keratosis found in smokers is also a well-known premalignant condition.

The oral mucosa can also be classified in terms of function, location or histology and can be divided into lining, masticatory and specialised mucosa.

Lining Mucosa

The oral surface of the lips, cheeks, floor of the mouth and ventral tongue are covered by a stratified non-keratinised epithelium. Deep to the epithelium lies the lamina propria where minor salivary glands are located. These glands become absent in the lips as the mucosa changes to keratinised skin at a junction called the vermillion border. Minor salivary gland tumours in the oral cavity are more likely to be malignant than benign from the outset, although the well-known pleomorphic adenoma ex carcinoma could be considered as a premalignant condition as it arises from a benign tumour.

Masticatory Mucosa

The attrition and friction that occurs on the masticatory mucosa requires a harder-wearing surface hence the need for keratinised epithelium. These surfaces include the gingivae and palate and are further strengthened by extensive interdigitation from the underlying lamina propria.

Specialised Mucosa

The epithelium of the tongue is complex. The thicker dorsal and lateral surfaces are keratinised, but not to the same degree as masticatory mucosa, and contain nerve endings for sensory and taste. The dorsal surface is unique with fungiform and circumvallate papillae which contain a lamina propria core.

Lips

The lip mucosa differs from the wet inner aspect, where minor salivary glands lubricate the surface, to the more exterior dry mucosa which lacks salivary glands and hence requiring licking to stay moist and to the outer dry mucosa which more resembles the skin.

The inner lip surfaces are covered with thick stratified squamous mucosa, whereas the dry outer surface is lightly keratinised. Long capillaries carry blood nearer to the surface hence the red appearance.

The lip is susceptible to oral and environmental carcinogens and is also a difficult surface to treat as it is not amenable to mouth rinses or many topical dermatological agents.

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Chapter 2
The Molecular Basis of Carcinogenesis

Carolina Cavalieri Gomes, Marina Gonçalves Diniz, and Ricardo Santiago Gomez

In this chapter, we will discuss the molecular basis of carcinogenesis. *First understand, and then treat!* Better treatment options for cancer and preventive approaches for potentially malignant lesions can be achieved only if the pathobiology of the disease is well understood. We have witnessed a shift in the therapeutic approaches to cancer, from "universal" therapies applied to several different tumour types to tailored and personalized treatment. Each tumour/lesion is unique. As the understanding of malignant transformation and carcinogenesis requires knowledge of molecular and tumour biology, we aim to discuss carcinogenesis initially in a broader context before discussing the effects of carcinogens on the aetiology of potentially malignant oral lesions.

Starting from the Beginning: Useful Concepts

Carcinogenesis Theories and Field Cancerization in Oral Epithelium

How does cancer arise? Is it merely a result of the accumulation of mutations over time? Is cancer a disease of the cell, or is it a disease of the tissue and of cell signalling in the microenvironment? There are several theories that attempt to explain the process of carcinogenesis by incorporating evidence and developing models [1].

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Among these theories are coherent non-exclusive models of carcinogenesis that focus on the biological changes in the epithelium alone, whereas other models also take the changes in the stroma into account. By far, the most widely disseminated carcinogenesis theory is the “somatic mutation theory” (SMT), which is based on the assumption that cancer is derived from a single somatic cell that accumulates DNA mutations. The SMT focuses on molecular changes in the epithelium. On the other hand, the “tissue organization field theory” (TOFT) considers carcinogenesis as a problem of tissue organization, highlighting the importance of stroma in the process of carcinoma formation [2]. There are strengths and weaknesses in both models, and they are not mutually exclusive in some areas; however, the TOFT carcinogenesis model has gained acceptance recently, as more scientific evidence has strengthened the importance of the microenvironment in tumour formation, demonstrating that cancer is a disease of the tissue and not simply a cellular disease.

Regardless of the carcinogenesis model chosen to explain how normal cells become cancer cells, one needs to consider basic concepts in human molecular genetics, as clinical and histopathological morphological changes are accompanied by molecular changes in tissues. Slaughter proposed in 1953 the field cancerization process in oral stratified squamous epithelium, showing that clinically normal tissue surrounding oral squamous cell carcinoma (OSCC) already harboured histopathological changes [3]. Interestingly, once the structure of DNA was solved, the field cancerization concept evolved and was updated, and it became known that clinical and morphological normal tissues surrounding OSCC had already incorporated molecular changes [4] (Fig. 2.1). An understanding of this concept is fundamental for those studying/treating OSCC and oral leukoplakia. The field cancerization in oral mucosa can be as large as 7 cm [5], which means that by removing an oral leukoplakia lesion, one cannot remove all cells that have been molecularly altered. This knowledge is also fundamental when interpreting research studies whose

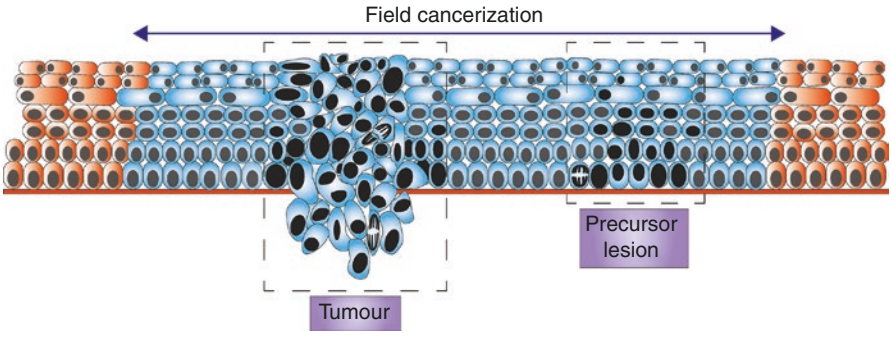

Fig. 2.1 Field cancerization. An area of epithelial cells harbouring molecular alterations (blue cells). A molecularly altered field can occur with normal histology, and in this figure we can observe a precursor lesion (oral leukoplakia) and an OSCC occurring in a same field cancerization

normal control reference tissues are “normal” tissues adjacent to the OSCC/oral leukoplakia.

Every pathology textbook describes the initiation and progression of cancer from a “clonal evolution” perspective. During clonal evolution, gradualism is assumed to occur, i.e. phenotypic features in cancers are believed to develop at a slow and continuous rate. According to clonal evolution, tumours are monoclonal, as they are derived from a single somatic cell, followed by the development of a neoplasm with cellular heterogeneity as a result of continued mutagenesis (we will discuss this topic in another section). When the tumour mass is established, clonal selection of the most well-adapted cells occurs, and the new, more fit clones rise to dominance and replace the entire population. This theory became the standard model of carcinogenesis and continues to spread, primarily because it is a simple and uncomplicated manner to explain a complex process. However, in this clonal evolution theory, even the definition of a “clone” is not unequivocal and straightforward and can be interpreted in more than one way [4]. Another caveat is that if cancers evolve linearly with time (gradualism), the malignant transformation of potentially malignant lesions, such as oral leukoplakia and Barrett’s oesophagus, should be predicted easily [6]. However, this phenomenon is not what happens in the clinic, as it is impossible to predict which “premalignant” lesions will evolve to become cancer.

Genetic progression models for oral leukoplakia have been proposed based on the somatic mutation carcinogenesis theory and on clonal evolution [5]. A monoclonal origin from OSCC associated with oral leukoplakia has been suggested, assuming that the carcinoma originated in the adjacent oral leukoplakia [7]. This hypothesis, however, is speculative, as retrospective studies using only the biopsy tissue from the excision of an OSCC lesion (including the adjacent oral dysplasia area) might not represent a true malignant transformation. OSCC is not always preceded by oral leukoplakia. To add a further layer of complexity to this subject, technological developments in genome analysis and mathematical and bioinformatics techniques have shown that the phenomena of punctuated and neutral evolution occurs during tumour evolution [6], and clonal evolution theory and gradualism fail to explain these findings. During the cancer evolutionary process, the genome is shaped not only by random mutations and non-random selection but also by random drift [4]. Both drift and selection change the frequency of alleles in a population, drift by random processes and selection based on *fitness*. Neutral evolution is defined as when selection is not operating and only the stochastic process of random mutations and drift occur. While random mutations and non-random selection have been the focus of several tumour evolution studies, random drift remains poorly understood, which does not allow for a complete understanding of how tumours evolve. A better understanding is yet to be obtained.

In the following sections, we will review briefly some basic concepts in human molecular biology. These definitions will help in following the discussions on cancer molecular pathogenesis.

DNA, RNA, Noncoding RNA, and Protein

The human genome is composed of DNA that contains approximately three billion base pairs distributed among 23 chromosome pairs (22 autosomal chromosomes and one sex chromosome). DNA molecules carry genetic information inside the cells and are composed of a double strand of linear polymers of nucleotides. DNA is packed inside the chromosomes in association with histone proteins, forming the nucleosomes. Each nucleosome consists of eight histone proteins around which DNA is wrapped [8], as shown in Fig. 2.2.

DNA is composed of the nucleotides adenine (A), cytosine (C), guanine (G), and thymine (T). It is organized into functional and physical units of heredity called genes. Genes have introns (regions which do not code for proteins) and exons (protein-coding sequences). The genetic DNA code is transcribed into mRNA, which is translated into proteins in that three nucleotides (codon) code for a specific amino acid in the protein (or are stop codons) [8].

Less than 2% of the human genome encodes proteins! Genetic sequencing of these protein-coding regions of the human genome is referred to as *whole-exome sequencing* (WES), and it is currently being used in biomedical research as well as

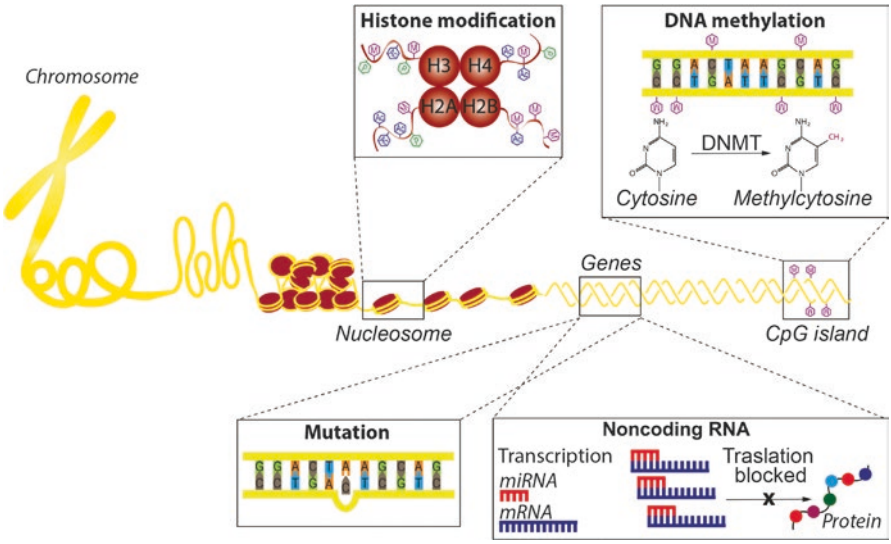

Fig. 2.2 DNA organization and carcinogenesis-related alterations. DNA is packaged in chromosomes forming complexes with histones. These complexes are the nucleosomes, and each nucleosome consists of eight histone proteins around which DNA is wrapped. Several alterations at nucleosome and nucleotide levels occur in carcinogenesis. The histone N-terminal tails modulate nucleosome structure and function and can suffer modifications, which include changes in their methylation and acetylation profiles. At nucleotide level, DNA mutations cause inactivation of tumour suppressor genes or activation of oncogenes. Gene expression levels can be altered by modifications in DNA methylation profiles (repressing transcription) or by ncRNA activity (repressing translation)

in the diagnosis of human diseases. Surprisingly, approximately 75% of the genome is transcribed into RNAs, including RNAs that have no protein-coding potential (noncoding RNAs) [9, 10]. Noncoding RNAs (ncRNAs) <200 nt are classified as small ncRNAs. Micro-RNAs (miRNAs) are a category of small ncRNAs. Conversely, ncRNAs >200 nt are classified as long ncRNAs (lncRNAs). While miRNAs are primarily involved in "silencing" gene expression (by targeting mRNAs) (Fig. 2.2), lncRNAs, which are more abundant than miRNAs in the human genome, exhibit a greater variety of functions in the regulation of gene expression [9].

miRNAs have been extensively studied in OSCC, and lncRNAs are in the process of being better characterized in such tumours [11, 12]. miRNA profiling in progressive and nonprogressive oral leukoplakias has shown that miR-21, miR-181b, and miR-345 increased expression in oral leukoplakias that progress to OSCC [13]. Additionally, higher expression levels of these miRNAs were found to be associated with cytological and histopathological parameters used to grade dysplasia, including an increased nuclear/cytoplasmic ratio and the presence of abnormally superficial mitosis [14]. LncRNA expression in oral premalignant lesions has been reported [15] but requires additional characterization and functional studies to better reveal the roles of such ncRNAs in the biology of these lesions.

Mutation and Genetic Variation

"There is no single sequence of the human genome." There are approximately three million sequence variations between any two unrelated persons, most of which do not have biological importance and do not contribute to physiological differences but do give rise to diversity between individuals.

Genetic variations that occur at a measurable frequency in the population are termed polymorphisms. A strict definition of a genetic polymorphism is variation present at a frequency ≥1% in the population. When a polymorphism is characterized by the substitution of a single nucleotide (e.g. the substitution of a C<T at a given position), it is defined as a single nucleotide polymorphism (SNP). Thousands of SNPs have been described, and there is a database of SNPs (and other short genetic variations) that can be accessed at https://www.ncbi.nlm.nih.gov/snp.

A mutation occurring in an exon (i.e. DNA that codes for proteins) can result in a change from one amino acid to another (missense mutation), a change that codes for a termination signal/stop (nonsense mutation), or no change in the amino acid (silent mutation). Mutations characterized by an insertion or deletion of one to a few nucleotides are called indels.

When DNA mutations are found in a given tumour, but not in peripheral blood/normal matching tissue, the mutation is considered a *somatic* mutation that originated in the tumour. However, if the mutation is also detected in normal constitutive DNA, it is classified as a *germline* mutation. An example of a germline mutation that predisposes individuals to cancer is the mutation in the *TP53* gene in Li-Fraumeni syndrome. However, the majority of tumours arise from somatic mutations and are

considered sporadic rather than familial tumours. Somatic mosaicism may occur, and a germline mutation cannot be detected in every constitutive normal cell; however, we will not discuss this topic in this review.

With the advances in next-generation sequencing (NGS) technology, the characterization of somatic genomic alterations in head and neck squamous cell carcinoma (HNSCC) is beginning to emerge. Recently, The Cancer Genome Atlas (TCGA) has profiled 279 cases of HNSCC by undertaking a comprehensive multiplatform characterization [16]. Similar to lung cancer and melanomas, HNSCC exhibits a high incidence of somatic mutations, which is consistent with its chronic exposure to mutagenic factors (tobacco smoking) [17]. Genes frequently mutated in HNSCC include *TP53*, *NOTCH1*, *HRAS*, *PIK3CA*, and *CDKN2A* [16]. *NOTCH1* gene mutations have been reported in a high proportion of oral leukoplakias and in OSCC, which raises the possibility of these mutations being important OSCC progression drivers [18].

Cell Cycle Differences Between Normal and Cancer Cells

Cell division occurs through sequential events that drive the progression from one cell cycle stage to the next, and it is altered in cancer cells [19]. The cell cycle is divided into two major phases, which are interphase and mitotic (M) phase. Interphase is subdivided into G1, S, and G2 phases. During G1, the cell grows and copies organelles; while in the S phase, the cell duplicates the DNA in the nucleus and in the centrosome. When the cell enters G2, it grows, synthetizes proteins and organelles, and prepares for mitosis. During the M phase, the cell separates its DNA and cytoplasm, leading to the formation of two cells.

Normal cells move through the cell cycle in a regulated manner, ensuring that they only divide when their DNA is not damaged and when there is room for more cells in the given tissue. The most important checkpoints that regulate the cell cycle are at the G1/S transition, the G2/M transition, and in the M phase. The cell cycle may be interrupted at any of these checkpoints so that the DNA can be repaired or that the cell can be eliminated by apoptosis.

Cyclins are one of the core cell cycle regulator proteins. Cyclins form complexes with cyclin-dependent kinases (CDKs), which in turn phosphorylate target proteins. There are several different cyclins, and the levels of each cyclin vary across the cell cycle, usually increasing only at the stage where they are required. Genetic mutations affecting cyclin or CDK genes can result in uncontrolled cell cycle progression. Cyclin D1, for example, is overexpressed in a variety of human cancers, including OSCC [20]. Conversely, there are CDK inhibitors that negatively control the cell cycle, including several different proteins such as p21, p16, p27, and p57. These proteins are frequently mutated or silenced by other mechanisms such as DNA methylation in human cancers. As CDKs play a central role in controlling cell cycle pathways, the development of therapeutic approaches to inhibit their kinase activity in cancer cells is currently in progress [21].

Alterations in the cell cycle include, but are not restricted to, genetic mutations (we will discuss this later in this chapter) and confer tumour cells with growth and survival advantages. While the normal cell cycle is regulated by proto-oncogenes, tumour suppressor genes, apoptosis genes, as well as DNA damage repair genes, in human neoplasia, these genes are usually dysregulated.

Oncogenes and Tumour Suppressor Genes

Oncogenes and tumour suppressor genes control cellular proliferation. An oncogene is a mutated form of a normal cellular gene referred to as a proto-oncogene. Proto-oncogenes are genes that positively regulate the cell cycle, and when they are over-activated by mutations, they are called oncogenes. This transformation of a proto-oncogene to an oncogene involves changes in protein amino acids, which can alter the protein structure. The mutations that convert proto-oncogenes to oncogenic alleles are named *activating mutations* to reflect "the gain of function". Additionally, proto-oncogene activation also can occur by gene amplification, in which extra gene copies are accumulated in the cell, resulting in extra protein production, or by chromosomal translocation (involving different mechanisms) [22].

Tumour suppressor genes are negative regulators of the cell cycle, and their functions are usually impaired in cancer. In contrast to proto-oncogene activating mutations, tumour suppressor genes usually harbour *loss-of-function* mutations with proteins that become functionally inactivated in cancer. Tumour suppressor genes normally control processes such as maintenance of genetic integrity, differentiation, cell-cell interactions, progression of the cell cycle, and apoptosis. Therefore, inactivation of tumour suppressor genes contributes to the disturbance of tissue homeostasis [23]. The most extensively studied tumour suppressor gene in human cancer is the *TP53* gene [24]. *TP53* prevents neoplastic transformation by temporarily or permanently activating the interruption of the cell cycle or by signalling cell death, and it is mutated in approximately half of all human cancer cases, including OSCC [16]. *TP53* is more frequently inactivated by small alterations, primarily by single nucleotide point mutations, and they occur at a higher frequency in hot spots that interfere with the functions of the encoded protein, which correspond to exons 5–8 of the gene.

Genetic Instability

Cancer cells commonly harbour defects in the mechanisms by which the genome is replicated and repaired and by which chromosomes are segregated during the cell cycle. These defects result in a higher rate of genetic alterations in cancer cells compared to normal cells and are less stable genetically than the surrounding normal tissue [25]. This genetic instability accelerates the occurrence of subsequent genetic

alterations; however, while genetic instability is a defect in a process, genetic alterations are stochastic events that do not necessarily indicate or cause genetic instability.

Genetic instability can be categorized into the following two major groups: instability at the nucleotide level and instability at the chromosomal level (chromosomal instability, CIN). Nucleotide-level instability includes deletions, insertions, and base substitution, while CIN refers to an increased rate of chromosome gains and losses, involving chromosomal missegregation due to mitotic errors [26]. A loss of specific chromosomal regions at constitutive heterozygous loci (loss of heterozygosity, LOH) that spans tumour suppressor genes has been reported to be a good predictor of malignant transformation of oral leukoplakia. Oral leukoplakias with LOH at chromosome regions 3p and/or 9p exhibited a markedly higher chance of malignant transformation compared to cases with 3p and 9p retention [27]. CIN involves cytogenetic changes that lead to changes in chromosome copy number, i.e. aneuploidy. Human cells contain 23 pairs of chromosomes and are diploid. A cell that has a number of chromosomes that is not a multiple of the haploid number is *aneuploid*. Aneuploid cells not only have a numerical abnormality but also commonly have chromosomal structural aberrations [26]. Aneuploidy occurs in a high proportion of solid human tumours, including OSCC [28]. In addition, as some OSCC arise in precursor lesions (potentially malignant oral disorders, including oral leukoplakia) and in preneoplastic epithelium, they can exhibit aneuploidy [29], and several studies have examined the possibility that aneuploidy indicates a risk of malignant transformation [30, 31]. Sperandio and co-workers [30] published a large series of DNA ploidy investigations in oral dysplasia, including 273 patients (32 with malignant transformation), for 5–15 years and demonstrated a positive predictive value for the malignant transformation by DNA aneuploidy of 38.5% [30]. In their study, the DNA ploidy status appeared to be correlated with epithelial dysplasia, and by combining both (ploidy status and dysplasia grading), the predictive value was higher than by using either technique alone. The utility of using DNA ploidy to predict the risk of oral dysplasia malignant transformations can vary according to the technique used, i.e. by flow or image cytometry [32].

While aneuploidy is a hallmark of several solid tumours, others do not show aneuploidy but rather exhibit defects in DNA repair. In a normal cell, DNA sequence errors arise as a result of mutagenic effects of environmental agents. In addition, errors caused by DNA polymerase arise during cell division (i.e. an endogenous form of mutagenesis). However, normal cells contain the machinery to repair these errors, as there are more than 100 known human DNA repair genes [33].

DNA repair pathways are classified into the following three functional categories: (1) direct reversal of DNA damage, (2) excision repair of DNA damage, and (3) DNA double-strand break repair. In the first pathway, a single enzyme repair system can restore the conformation of pyrimidines after UV light damage in a relative simple light-dependent reaction. The second pathway is composed of the following three different repair systems: base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) genes. BER proteins excise and replace

a single base and are commonly used to repair damage caused by insult to endogenous DNA (such as in response to oxidative DNA damage). NER excises oligonucleotides in response to genomic damage caused by UV exposure and involves at least 30 different proteins. MMR, the third excision repair system, preserves genomic integrity by acting in cases that involve inaccuracy in DNA replication. In the occurrence of a mutation during DNA replication, MMR recognizes and excises the mismatched nucleotide, resynthesizes DNA, and then ligates the broken strand. In addition, a direct reversal of DNA damage and excision repair of DNA damage can be repaired by a third pathway, which involves the repair of double-stranded DNA. This pathway uses a number of proteins to repair double-stranded DNA breaks (DSBs) that result from exogenous and endogenous agents, including ionizing radiation, chemical exposure, and somatic DNA recombination [33].

All of these mechanisms of DNA damage repair are interconnected and act cooperatively to maintain genome integrity. However, in cancer, these repair systems may be impaired. Mutations or loss of function of these genes may result in a reduced capacity for the correction of DNA errors, thereby predisposing the cell to genomic instability. If the functions of these genes are impaired, then the cell cannot repair the DNA, and programmed cell death can be triggered following the activation of apoptotic genes.

Evasion of Apoptosis

Tumour growth results not only from increased cell division, but it also depends on preventing cells from entering apoptosis. Neoplastic cells have the capacity to evade apoptosis by several mechanisms, enabling them to increase in number. These apoptosis-evasion mechanisms include the amplification of anti-apoptotic machinery, downregulation of the pro-apoptotic program, or both [34, 35]. There are several examples of altered regulation of genes that encode either the anti-apoptotic or pro-apoptotic Bcl-2 family in cancer. The *BCL-2* anti-apoptotic gene was first described because of its translocation in non-Hodgkin lymphomas, and it is also amplified in other tumour types [34]. Another mechanism that can lead to the overexpression of BCL-2 is the loss of micro-RNAs that repress BCL-2 gene expression, as observed in chronic lymphocytic leukaemia, in which micro-RNA 15 and 16 genes are deleted [10].

Immunotherapy and Immune Escape

The microenvironment is a critical regulator of tumour biology and can either inhibit or support malignant transformation and tumour development, growth, invasion, and metastasis. One important component of the tumour microenvironment is the immune system. Tumour cells express antigens that can mediate their

recognition by host CD8+ T cells and allow clinically detected tumours to evade antitumour immune responses.

Immunotherapy is an old concept, which has recently gained increased attention from the scientific community. These strategies are designed to alter the immune system, either by stimulating the patient's own immune system to attack cancer cells or by providing "immune system man-made components" such as proteins. Unfortunately, not all tumours respond to immunotherapy, and to increase the efficacy of immunotherapy, the immune escape mechanisms used by cancer cells must be overcome. Tumour cells can evade immune elimination by different mechanisms, such as the loss of antigenicity and/or the loss of immunogenicity, and by establishing an immunosuppressive microenvironment [36]. Immunotherapy is beginning to be explored in the oral cancer scenario, but the majority of novel immunotherapeutic strategies are currently investigational [37].

Epigenetics: Changes Beyond Genetic Sequence Changes

It is common to consider cancer a "genetic" disease. However, genetics and epigenetics cooperate in cancer development and progression. There is crosstalk between the genome and the epigenome. Genetic alterations of the epigenome contribute to cancer, and additionally, epigenetic processes can cause point mutations and disable DNA repair [38]. Epigenetics is defined as "heritable changes in gene expression that are not accompanied by changes in the DNA sequence". If we are not strict with the "heritability", noncoding RNAs can be considered epigenetic modifiers, and they have been discussed previously in this chapter. However, the most important epigenetic modifiers in cancer are DNA methylation, histone modification, and chromatin remodelling.

DNA methylation is classically associated with gene silencing, although other functions have recently been described. It occurs on cytosine, which is converted to 5-methylcytosine by the action of DNA methyltransferase (DNMT) enzymes (Fig. 2.2). Frequently, the altered C is adjacent to a G, and methylation is distributed in CpG sequences throughout the genome. CpGs are clustered in CpG islands, often at gene promoters (i.e. at the start of genes, where transcription machinery binds) (Fig. 2.2). CpG islands tend to be unmethylated, and when methylation occurs in CpG islands, it results in silencing of gene expression. DNA methylation can lead to gene silencing by different mechanisms that involve the physical impediment of transcriptional proteins binding to the gene and the indirect alteration of chromatin structure, forming heterochromatin. Heterochromatin is a compact and inactive form of chromatin. In cancers, the earliest epigenetic aberration found was a genome-wide hypomethylation [38]. Head and neck squamous cell carcinoma (HNSCC) exhibits global genomic