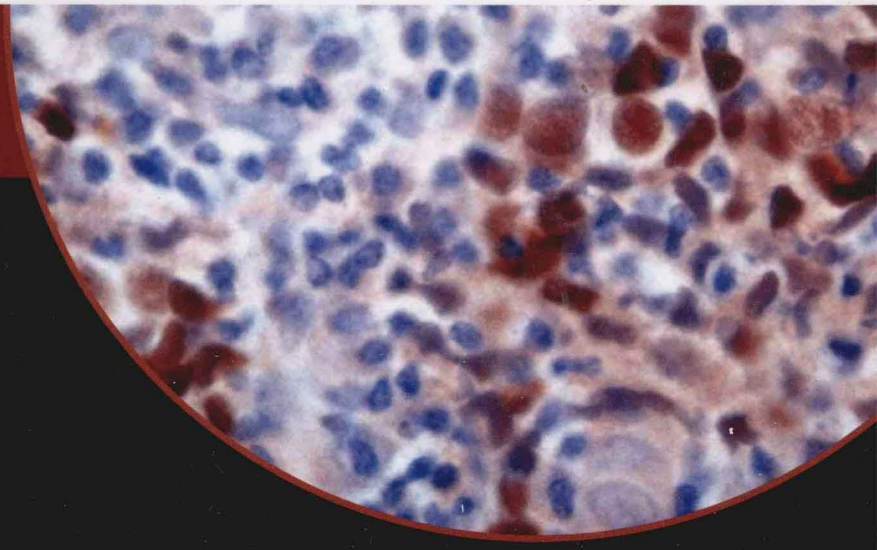

Early Diagnosis and Treatment of Cancer

HEAD and NECK CANCER

EDITED BY
Wayne M. Koch

SERIES EDITOR
Stephen C. Yang

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EARLY DIAGNOSIS AND TREATMENT OF CANCER

Series Editor: Stephen C. Yang, MD

Head and Neck Cancer

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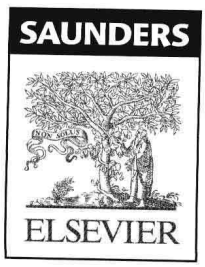

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EARLY DIAGNOSIS AND TREATMENT OF CANCER: HEAD AND NECK CANCER
ISBN-13: 978-1-4160-5202-9

Library of Congress Cataloging-in-Publication Data

Early diagnosis and treatment of cancer : head and neck cancer / edited by Wayne M. Koch.
p. ; cm.—(Early diagnosis and treatment of cancer series)
ISBN 978-1-4160-5202-9
1. Head—Cancer. 2. Neck—Cancer. I. Koch, Wayne M. II. Title: Head and neck cancer. III. Series: Early diagnosis and treatment of cancer series.
[DNLM: 1. Head and Neck Neoplasms—diagnosis. 2. Early Diagnosis. 3. Head and Neck Neoplasms—therapy. WE 707 E12 2010]
RC280.H4E27 2010
616.99'491—dc22

2009028019

Acquisitions Editor: Dolores Meloni
Design Direction: Steven Stave

Printed in China.

Last digit is the print number: 9 8 7 6 5 4 3 2 1

Series Preface

Seen on a graph, the survival rate for many cancers resembles a precipice. Discovered at an early stage, most cancers are quickly treatable, and the prognosis is excellent. In late stages, however, the typical treatment protocol becomes longer, more intense, and more harrowing for the patient, and the survival rate declines steeply. No wonder, then, that one of the most important means in fighting cancer is to prevent or screen for earlier stage tumors.

Within each oncologic specialty, there is a strong push to identify new, more useful tools for early diagnosis and treatment, with an emphasis on methods amenable to an office-based or clinical setting. These efforts have brought impressive results. Advances in imaging technology, as well as the development of sophisticated molecular and biochemical tools, have led to effective, minimally invasive approaches to cancer in its early stages.

This series, *Early Diagnosis and Treatment of Cancer*, gathers state-of-the-art research and recommendations into compact, easy-to-use volumes. For each particular type of cancer, the books cover the full range of diagnostic and treatment procedures, including pathologic, radiologic, chemotherapeutic, and surgical methods, focusing on questions like these:

- What do practitioners need to know about the epidemiology of the disease and its risk factors?
- How do patients and their families wade through and interpret the many tests they face?
- What is the safest, quickest, least invasive way to reach an accurate diagnosis?
- How can the stage of the disease be determined?
- What are the best initial treatments for early-stage disease, and how should the practitioner and the patient choose among them?
- What lifestyle factors might affect the outcome of treatment?

Each volume in the series is edited by an authority within the subfield, and the contributors have been chosen for their practical skills as well as their research credentials. Key Points at the beginning of each chapter help the reader grasp the main ideas at once. Frequent illustrations make the techniques vivid and easy to visualize. Boxes and tables summarize recommended strategies, protocols, indications and contraindications, important statistics, and other essential information. Overall, the attempt is to make expert advice as accessible as possible to a wide variety of health care professionals.

For the first time since the inception of the National Cancer Institute's annual status reports, the 2008 "Annual Report to the Nation on the Status of Cancer," published in the December 3 issue of the *Journal of the National Cancer Institute*, noted a statistically significant decline in "both incidence and death rates from all cancers combined." This mark of progress encourages all of us to press forward with our efforts. I hope that the volumes in *Early Diagnosis and*

Treatment of Cancer will make health care professionals and patients more familiar with the latest developments in the field, as well as more confident in appling them, so that early detection and swift, effective treatment become a reality for all our patients.

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Preface

Early detection of cancer is one of the "Holy Grails" of management of that disease, made all the more urgent by the recent announcement that cancer is expected to supersede heart disease as the number one cause of death in the United States by 2010. The fundamental concept is based on the time-honored observation that treatment is most successful with earliest staged lesions. Together with the concept that tumors grow and increase in stage in a generally uniform manner over time, clinical experience of improved outcome when cancer is detected early provides enormous intuitive benefit to early detection. Whether this logic follows the observations is arguable, but the rationale is strong enough to drive many clinical and laboratory endeavors, which we outline in the chapters that follow.

Endeavors to improve early detection of head and neck cancer face some challenges common to all types of cancer and some that are particular to upper aerodigestive malignancy. From a population and demographic vantage point, head and neck cancer is much less common than the most common adult malignancies—lung, prostate, breast, and colon—but it ranks in the top ten in the United States, about as common as lymphoma or melanoma. Elsewhere, head and neck cancer is a bigger public health problem. Long the most common adult cancer in India, the emerging economy in China has opened that population to the risk of smoking-related cancers including HNSCC. It is too common a cancer to ignore with no effort at public health endeavors for detection and prevention, but uncommon enough to raise problems with who to screen and how to raise and maintain health provider skill and awareness for the task. The dental community has embraced head and neck cancer screening through useful efforts of the American Dental Association and the National Institute of Dental and Craniofacial Research, and, indeed, for those Americans who visit the dentist regularly, dental screening of soft tissues makes sense. Dental providers look at the mouth routinely, gaining a familiarity with normal and diseased states, and they see their clients frequently enough to maintain a regular program of screening. However, those most at risk for traditional forms of head and neck cancer are heavy smokers and drinkers, a group that often does not visit the dentist regularly. Furthermore, dental screening ends at the tonsil arch, where a new epidemic of cancer affecting nonsmokers and attributed to the human papillomavirus begins. Therefore, despite the advent of several new products marketed to help dental professionals with oral screening (which can be billed to the cash-paying client at a markup), dental screening alone will not adequately address all early detection concerns for head and neck cancer.

Primary care providers can visualize the tissues of the oral cavity and can palpate the neck for metastatic nodes, but all other regions of the upper aerodigestive tract are inaccessible for screening by visualization or palpation except by expert clinicians, chiefly otolaryngologists. Hence, like colon cancer, head and neck cancer screening is relegated to a specialist-referral arrangement outside the routine of health maintenance paradigms. Unlike prostate cancer, there is no blood test that adequately screens for head and neck cancer, and individuals cannot perform effective self-exams as they can for breast cancer. Even for the specialist, small cancers deep within crypts of the lympho-epithelium of the oropharyngeal tonsillar tissue (palatine and lingual

tonsils) or within hidden folds in the hypopharynx or laryngeal ventricle may remain elusive for years before suspected and detected.

Turning to technology, hope for an effective screening tool for head and neck cancer has been the impetus for a great deal of research during the past decade, much of which is outlined in this volume. The challenge to identify markers for cancer that can be detected quickly, noninvasively, and cheaply with high specificity and sensitivity is daunting. Even if such a test were available, individuals at risk would still need to be reached by the health care establishment in order to receive the test, and then a clinically detectable lesion would need to be identified prior to initiating intervention. Still, efforts to apply radiographic studies, molecular detection of tumor-specific proteins, or nucleic acid alterations examining various specimens, including both blood and saliva, are underway across the country and around the world.

Much of this volume is dedicated to treatment of early cancers of the head and neck, where the goal is to effectively eradicate cancer while preserving both function and form of vital tissues and organs. Each region within the upper aerodigestive tract is unique in its challenges in this regard. Small lesions within the larynx are amenable to laser excision with great precision, avoiding collateral damage to remaining portions and preserving laryngeal function. Oral cavity lesions, likewise, are accessible to simple surgical extirpation, often with acceptable functional result. However, in both these regions, occult changes within cells surrounding the clinical lesion lead to a high rate of recurrence of cancer over time, particularly in the smoking population. Cancers of the nasopharynx and oropharynx are more sensitive to radiation, making this the treatment of choice after early detection. However, radiation delivered to these areas is a one-time tactic, making management of those cases that are not controlled a much greater strategic problem. Salvage surgery in these areas is hampered by the need to detect persistence early in a treatment-altered field even more limited in its access to physical and radiographic screening.

This timely volume seeks to address these issues in a comprehensive manner, pointing out the need, the challenges, and the prospects for future innovation. With cancer on the ascent in public concern in the United States, and increasing in frequency along with tobacco use in major population centers worldwide, it is difficult to overestimate the importance of early detection. It is our hope that the thoughtful submissions that follow will contribute in coming years to endeavors to improve the plight of many individuals who contract this most debilitating and deadly disease.

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Contents

1 Molecular Gene Alterations as Early-Detection Markers

Ian M. Smith, Joseph A. Califano III, and Patrick K. Ha

KEY POINTS

- Early diagnosis of head and neck squamous cell carcinoma (HNSCC) is the most significant factor in predicting survival for each tumor site.
- HNSCC has many promising molecular markers including human papilloma virus (HPV), p53, cyclin D1, p16, cyclooxygenase-2 (COX-2), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF).
- Molecular markers have been applied in several ways: early detection of cancer or screening and disease follow-up and progression.
- Population-based screening tests are extremely difficult to successfully undertake, owing to the low overall incidence and the associated rates of false-positive results in this sample set.
- Alternative efforts for molecular detection that do not require as much specificity for clinical use include molecular surgical margin analysis, lymph node analysis, disease surveillance after treatment, molecular staging, and/or molecularly tailored therapies.
- To date, few of the approaches for molecular screening and diagnosis have been taken to clinical trials. Notable exceptions are tests for loss of heterozygosity (for prognosis of premalignant lesions) and toluidine blue (for early detection).
- Many detection sources have been studied including saliva, salivary rinses, mouth scrapings, and blood serum or plasma.
- Major developmental efforts have been conducted on specific alterations including p53 mutation detection, mitochondrial mutations, promoter hypermethylation, loss of heterozygosity, and HPV detection.

Introduction

Early diagnosis is the most significant factor in predicting survival for each tumor site (Table 1-1). With the advent of newer biomedical technologies, there has been an increased interest in the development of early screening tests and noninvasive diagnostic tests for head and neck squamous cell carcinoma (HNSCC). Basic science understanding of the genetic and epigenetic alterations in the pathogenesis of cancer has yielded new molecular diagnostic approaches. Our understanding of tumor molecular biology has led to translating these advances into relevant clinical situations and applied pathology. Many promising molecular markers have been found including human papilloma virus (HPV), p53, cyclin D1, p16, cyclooxygenase-2 (COX-2), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF). This chapter discusses HNSCC molecular biomarkers—their benefits and limitations.

Molecular markers have been applied in several ways: early detection of cancer, disease follow-up and progression, specialized applications such as molecular surgical margin and lymph node analysis, molecular staging, and selection of tailored therapies. Each use of molecular markers is intended to improve patient survival, but these markers do so in different ways.

Table 1-1. Five-year Survival of Head and Neck Squamous Cell Carcinoma by Site and Extent of Disease at Presentation

	Extent of Disease		
Tumor Site	**Local**	**Regional**	**Distant**
Lip	91.4	82.6	52.2
Oral cavity	71.4	45.8	21.8
Salivary gland	85.5	56.7	23.4
Oropharynx	58.4	41.2	20.3
Nasopharynx	65.3	50.9	28.8
Hypopharynx	46.8	29.7	15.7
Larynx	79.2	54.8	35.4
Other	61.8	38.4	11.6

Adapted from Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP: Trends in incidence and prognosis for head and neck cancer in the United States: a site-specific analysis of the SEER database. Int J Cancer 114(5):806–816, 2005.

This chapter discusses applications for molecular markers, premalignant disease and cancer progression model, body fluids, and mechanisms for detection, and it gives a background of genetic alterations used for detection. Several underlying assumptions should be mentioned at the outset. Tumor development is now widely recognized as a process involving multiple alterations affecting the genetic code that accumulate over time. Key molecular pathways may be affected by alterations in one of several targets, which may accrue over time in an order that may vary from case to case. There are several recognized clinical subsets of head and neck cancer, such as HPV-related oropharyngeal cancer, typical smoker-drinker cancers of the oral cavity or larynx, nonsmoker, nondrinker cancers of the lateral tongue, and so on. Each of these subsets may have somewhat distinctive molecular profiles.

The use of molecular markers for early detection therefore depends on the relative prevalence of specific alterations in the population to be tested and on the relative position of each alteration in the tumor progression pathway. Interpretation of collected molecular data must take into consideration several issues. First, is the molecular target truly indicative of the fully transformed malignant state, or by itself is it indicative only of risk or probability of cancer? Some markers may be present to a greater degree in cancer but still present in benign states. If so, how distinct is the breakpoint between benign and malignant state?

Molecular Marker Applications

Early Detection (Screening)

Head and neck squamous cell tumors have a tendency toward late-stage presentation because these growing neoplasms are often asymptomatic and taken as a whole. The noted exception is laryngeal lesions, which can often arise on the vocal folds and cause hoarseness, leading to an earlier medical presentation as well as anterior oral cavity lesions that may be easily visualized by the patient or the primary care or dental professional. Other head and neck cancers manifest as dysphagia, odynophagia, or a mass—symptoms associated with late-stage presentation. This chapter covers areas of investigation in molecular diagnostics, which include tests that may be adapted to screen high-risk populations without previous symptoms or findings, and the development of tests that may be used for detection of occult, persistent, or recurrent disease in patients who have already been diagnosed with HNSCC.

Within the realm of early detection, population-based screening tests are extremely difficult to successfully develop and deploy, owing to the low overall incidence and the associated rates of false-positives in this sample set. Population-based screening tests do benefit from well-outlined risk factors including tobacco and alcohol use. Even so, they suffer because of the difficulty of producing tests with adequate sensitivity to be useful in detection and with adequate specificity to not generate large numbers of false-positive results in the setting of low incidence. Furthermore, it is known that many of the common genetic alterations in HNSCC can be detected in patients who smoke but who do not have evidence of overt carcinoma. Other molecular detection applications, such as molecular margin detection, detection of nodal metastasis, and disease surveillance after treatment, simply do not require this level of testing specificity because the pertinent markers can be identified from the analysis of existing tumor tissue.

Tests developed in the laboratory for molecular diagnosis must be validated in clinical trials. To date, few of the approaches for molecular screening and diagnosis have been taken to clinical trials. Notable exceptions are tests for loss of heterozygosity (LOH) used for prognosis and toluidine blue staining of oral lesions as a means of enhancing detection.

Disease Surveillance: Follow-up and Progression

Routinely, patients who are postoperative or post-chemotherapy or radiation therapy are monitored by interval physical examination and radiologic imaging. Identifying early recurrence would be expected to have survival benefit or at least a benefit in reduced morbidity and tumor burden in patients with recurrences. Many of the molecular techniques discussed here lack the requisite sensitivity and specificity for population-based screening. However, because primary tumors can be studied directly in the setting of disease surveillance for recurrence, molecular alterations can be directly tailored to the patient. These efforts show remarkable promise in monitoring recurrence and treatment outcomes. Prominent examples of techniques include toluidine blue and LOH.[1]

Molecular Margin or Lymph Node Analysis

Traditional intraoperative frozen section and paraffin sections have been used to detect the presence of negative surgical margins and lymph node metastasis. However, intraoperative frozen sections are expensive, time-consuming, and effort-intensive and may not be as accurate as nonfrozen histopathology. Therefore, some surgeons rely on postoperative reports of margin detection based on hematoxylin-and-eosin (H&E)–stained slides to guide therapy. If margins are positive, patients may be subjected to repeat operations. Quick, reliable, and sensitive molecular detection techniques would augment surgical management. Published studies have used p53 mutation detection, p53 expression, and methylation markers to assess the presence of positive margins with varying success.[2,3]

Nodal status has a significant impact on overall survival in HNSCC, underscoring the importance of accurate staging of cervical lymph nodes. The traditional diagnostic approach relies on H&E staining; however standard methods of pathologic examination can yield false-negative results. Often, isolated neoplastic cells or micrometastases can be missed.[4] Investigators have attempted to use techniques such as quantitative reverse transcription-polymerase chain reaction (qRT-PCR), to detect cancer-specific antigens fast enough for use with pathologic frozen-section analysis. No studies have shown adequate usefulness for wide-scale adoption in detecting tumor spread to lymph nodes. Yet, pilot scale studies have shown promise in improv-

ing nodal staging. Molecules used successfully for detection include pemphigus vulgaris antigen[5] and squamous cell carcinoma antigen.[6] These studies offer promise that molecular detection will be translated into the clinic. One significant caveat remains that the clinical significance of molecular-positive cervical metastasis has not been demonstrated to date. The identification of tumor in a pathologically N0 neck, resulting in upstaging to N+ would be expected to have a greater impact than identification of additional nodes in cases already pathologically N+. Carefully designed prospective trials are a must to prove the efficacy of these applications.

Molecular Staging and Molecularly Designed Therapies

Efforts to coordinate clinical trial treatment arms to molecular diagnostic criteria have been made in the last 10 years. Adequate predictors of response have been elusive, but some promising targets have been found. One group has found that cyclin D1 overexpression correlates with cisplatin sensitivity in cell lines.[7] Overexpression of epidermal growth factor receptor (EGFR) has also been implicated in cisplatin resistance.[8]

Cancer Progression Model

Approaches to molecular diagnosis rely on heritable, documented changes involved in the pathogenesis of cancer. The head and neck cancer progression model was initially derived from Vogelstein's description of colon cancer progression.[9] This theory states that cancer results from multiple accumulated, progressively transforming genetic alterations in clonal population cells. This hypothesis is based on several principles, including the following: (1) neoplasms are caused by tumor suppressor gene inactivation and/or proto-oncogene activation; (2) an accumulation of genetic and epigenetic events causes the development of a tumor phenotype; and (3) net accumulation of alterations rather than a specific order of events determines the malignant phenotype.[10,11] In oral cancer, a similar stepwise progression model has also been described.

HNSCC is highly correlated with environmental exposures such as cigarette smoke, smokeless tobacco, and alcohol. Most theories concerning the etiology of HNSCC derive from accumulated molecular changes that are inflicted by DNA-damaging, carcinogenic exposures. Through comparison of the spectrum of alterations in premalignant and invasive cancers, genetic and epigenetic alterations can be classified as early or late.

Several common clinical lesions that may represent the premalignant state leading to HNSCC are leukoplakia, erythroplakia, oral lichen planus, and submucous fibrosis. Clearly an elevated cancer risk is associated with these lesions, but controversy remains regarding whether cancers develop from these lesions directly or are merely heralded by their appearance with invasive tumor and eventually appear elsewhere in the upper aerodigestive tract. Predicting which lesions progress and also being able to detect these lesions early can help in early disease eradication. Several published reports indicate that dysplastic leukoplakic lesions can be stratified for their ability to develop into cancer, based on allelic LOH.[12,13] Molecular analysis may indicate the likelihood of progression of both free-standing dysplastic lesions and those that remain after previous treatment of invasive cancer. Reliable prediction of cancer development in these lesions is discussed in several sections of this chapter.

Mechanisms of Detection

Sample Collection

To date, samples for molecular detection have primarily come from oral fluids (brushings, washings, or saliva) or blood (plasma or serum). Unlike with cervical cancer,

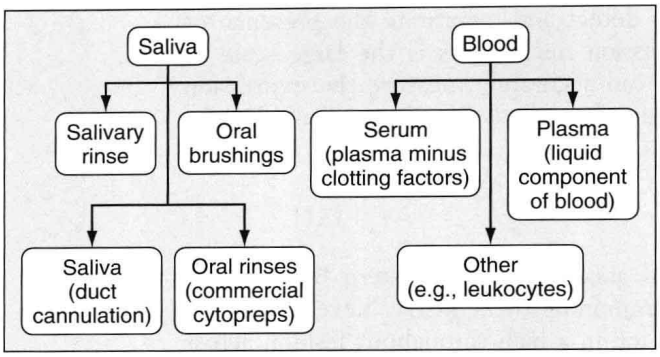

Figure 1-1. Body fluid compartment sources for diagnostic and screening studies in head and neck squamous cell carcinoma (HNSCC).

traditional pathologic approaches (e.g., cytology) have failed to yield adequate test sensitivity and specificity for clinical usefulness. Figure 1-1 shows different methods of sample collection.

Blood

Blood is a convenient source of DNA for molecular diagnostic efforts to find genetic and epigenetic alterations that are cancer-specific. Studies use either the *blood plasma*—the liquid component of blood, or the *serum*—blood plasma in which clotting factors (e.g., fibrin) have been removed. Other studies have considered changes in *blood leukocytes*, such as oxidative 8-oxoguanine DNA damage to predict cancer risk and treatment response.[14]

Oral Cavity

Several collection methods exist for surveying tumor-associated molecular changes from the oral cavity. *Salivary rinses* refer to sample collection in which a patient rinses the mouth with saline or other liquid and simply expels it into a specimen cup. Saliva can be collected by repeated expectoration over time, from the gingival pocket, or from salivary duct cannulation. Usually, centrifugation is used to produce fluid devoid of cells and debris. Finally, commercially available cytology kits harvest cells using *oral brushings*. Brushes used in suspicious areas to scrape oral mucosa yield markedly more cells than do rinses.

Assays

DNA Techniques

Molecular assays depend on the specific requirements of the cellular molecule to be detected: DNA, RNA, or protein. Classic techniques for DNA detection include big dye termination sequencing, polymerase chain reaction (PCR), quantitative real-time PCR (qPCS), and in situ hybridizations. In addition, epigenetic alterations (e.g., promoter methylation) can be detected by bisulfite sequencing, methylation-specific PCR (MSP), or in a quantitative fashion by quantitative methylation-specific PCR (qMSP).

RNA Techniques

RNA detection can be accomplished by RT-PCR or by quantitative RT-PCR, or the entire mRNA complement can be detected with an expression microarray. Expression microarrays use small silicon chips or glass slides with embedded

short segments of DNA (termed *oligos*) to detect and quantitate the presence of specific sequences. The advantage of expression microarrays is the large scale of screening afforded. Chips are devised that can accurately measure the expression of all 40,000 plus genes in the genome. The disadvantage is the statistical and logistic management of such a large quantity of data.

Protein Techniques

Proteins have been detected by traditional assays such as Western blot, ELISA (enzyme-linked immunosorbent assay), and immunohistochemistry. Novel proteomic techniques have the ability to assay expression in a high-throughput fashion across the entire human genome. New discovery techniques include two-dimensional SDS-PAGE gels (separates proteins based on size, charge, and isoelectric point) along with innovations in mass spectography, SELDI-TOF (surface-enhanced laser desorption and ionization time-of-flight mass spectrometry), and MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight mass spectrometry). These proteomic techniques can assay expression of a large number of different proteins, and efforts are being made to apply these to samples such as saliva and blood for molecular cancer detection.

Specific Genetic Alterations

Cytogenetic Alterations: CGH/FISH/SKY

HNSCC displays many cytogenetic alterations including aneuploidy, chromosomal gain, chromosomal loss, and translocations. Several techniques exist to assess the presence of cytogenetic alterations: spectral karyotyping (SKY), which is a chromosomal staining technique that is helpful in finding translocations, and fluorescence in situ hybridization (FISH), which uses probes designed to detect specific copy number changes in chromosomes. These techniques allow for the detection of large-scale genetic alterations or rearrangements that may not be detected using other molecular assays. Figure 1-2 shows one type of well-studied cytogenetic alteration in head and neck cancer: LOH, in which there is cytogenetic or genetic loss of one allele.

Others have considered whole-chromosomal alterations, specifically aneuploidy. Sudbo and colleagues[15] in 2001 showed that chromosomal copy changes (aneuploidy) could have prognostic significance in HNSCC. Sudbo's group found a significant association between cancer progression in premalignant lesions and chromosomal aneuploidy. This work[15] has been retracted because of data inconsistencies. At this

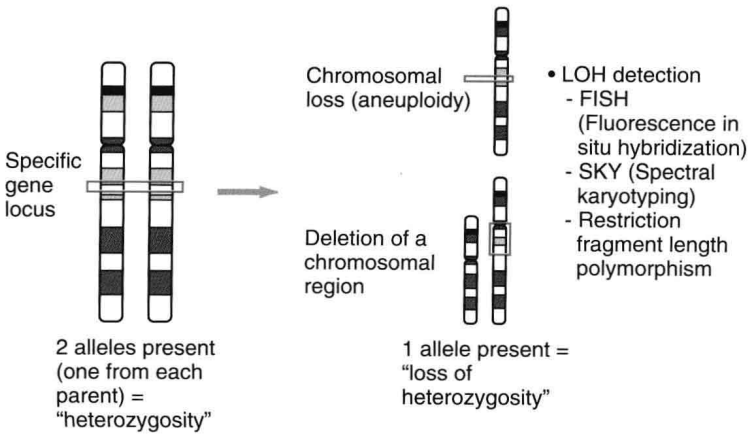

Figure 1-2. Loss of heterozygosity (LOH). LOH refers to loss of one parental allele that usually harbors a tumor suppressor gene. This forms half of the canconical Knudson two-hit hypothesis.

time, further work is being conducted to investigate whether there is any valid effect of chromosomal aneuploidy on the progression of premalignant lesions.

PCR–Based Detection: Loss of Heterozygosity/Microsatellite Instability

Microsatellite instability (MSI) refers to alterations in copy number of small repeats of a short nucleotide motif (usually one to five nucleotides long) in the genetic sequence of a cell. It was discovered initially in colon cancer,[16] but also has been found to be a feature of head and neck cancer.[17] Figure 1-3 demonstrates how MSI is detected. The most common microsatellite in humans is a dinucleotide repeat of cytosine and adenine, which occurs in tens of thousands of locations in our genome. When MSI is present, these areas are aberrantly replicated, leading to expansion or contraction of the locus. MSI has been found to be associated with errors in DNA replication and DNA repair enzymes.[18] After the discovery of MSI, efforts were made to use these microsatellite alterations to detect cancer cells in a background of normal tissue. Microsatellite analysis by PCR can reveal either MSI or LOH (loss of one portion of a parental chromosomal). LOH is one possible mechanism of tumor suppressor gene inactivation fulfilling Knudson's two-hit hypothesis.

According to Knudson, complete silencing of a suppressor gene occurs by inactivation of each allele through a variety of mechanisms. Researchers have used these tumor-specific alterations for cancer detection using saliva or plasma of HSNCC patients. A group of MSI alterations was initially reported in the serum of 29% of patients with HNSCC.[19] These alterations were then used to assay saliva samples of patients with HNSCC in a pilot study to detect tumor-specific genetic alterations in exfoliated oral mucosal cell samples. Spafford and colleagues[20] studied samples from 44 HNSCC patients and 43 healthy control subjects. They showed LOH or MSI in at least one marker in 38 (86%) of 44 primary tumors with identical alterations found in the saliva samples in 35 of the 38 cases (92% of those with markers; 79% overall). MSI was detectable in the saliva in 24 of 25 cases (96%) in which it was present in the tumor; LOH was identified in the test sample in 19 of 31 cases (61%) in which it was found in the primary tumor. No microsatellite alterations were detected in any of the samples from the healthy control subjects.[20]

Microsatellite testing is now clinically available. However, other studies have found greater rates of background MSI in normal samples, which has mitigated the sensitivity in widespread application of these tests.[21]

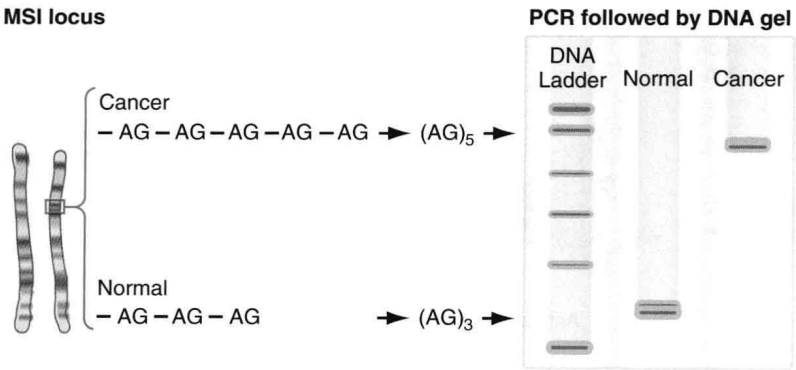

Figure 1-3. Microsatellite instability (MSI). Polymerase chain reaction (PCR) is used to amplify a sequence with known MSI. Typically, the alteration is detected by standard DNA gel electrophoresis, and the difference in the length of the MSI is seen as difference in migration of the band. In this example, the MSI in the cancer has resulted in an increase in the number of AG repeats and a longer DNA fragment that migrates less through the gel electrophoresis.