Literature Review

PennWell

GRAND ROUNDS
in Oral-Systemic Medicine™

THE MOLECULAR/GENETIC BASIS OF ORAL CANCER

Patrick K. Ha, MD†

Abstract
Oral squamous cell carcinoma (OSCC) represents a significant percentage of new cancer cases worldwide, and is the sixth most common cancer. In the United States, survival from OSCC has improved, but 25% of patients still succumb to their disease at some point, despite optimal surgical therapy and adjuvant treatment. Environmental factors clearly play a role in the development of premalignant disease and oral cancer. The conversion of normal mucosa into cancer is accompanied by many different alterations in the deoxyribonucleic acid (DNA) makeup of these lesions. The detection of DNA changes, even in precancerous conditions, has led to the development of a multistage model of carcinogenesis. We will provide an overview of the genetic alterations thought to be involved in OSCC and their possible clinical implications, including the development of screening techniques and therapeutic interventions.

Citation: Ha P. The molecular/genetic basis of oral cancer. *Grand Rounds Oral-Sys Med.* 2007;1:20-28. (Digital version *Grand Rounds Oral-Sys Med.* 2007;1:20-28.)
*(A complimentary copy of this article may be downloaded at **www.thesystemiclink.com**.)*

Key Words: Genomics, oral cancer, molecular cell biology, oral carcinoma

Introduction

OSCC represents the sixth most common cancer worldwide and is eighth in the United States. The use of tobacco and tobacco-related products, as well as alcohol, is the cause in up to 90% of cases. Other environmental exposures such as betel quid, mate, areca nut, and human papilloma virus have also been implicated as etiologic factors. Thus, the worldwide incidence has remained relatively steady because of a lack of public health awareness of health consequences, especially in developing countries where smoking is more prevalent.

The development of oral cancer is thought to occur in a stepwise fashion — lesions often begin as a leukoplakia, or a white patch, potentially indicative of premalignant disease. The cellular characteristics are what define the risk of progression, and these lesions can demonstrate mild, moderate, or severe dysplasia, which then can evolve to a carcinoma in situ, and ultimately to an invasive cancer. Screening and surveillance by trained dentists and physicians is imperative because of the progressive nature of the disease and its location, especially in high risk individuals.

Treatment usually involves biopsy of any suspicious lesion, followed by surgical excision if necessary. Many of these lesions can be detected and successfully treated in their early stages because of the ready access to tissue. Depending on the thickness or size of the lesion, management of the regional cervical lymphatic basins may also be necessary. Adjuvant radiation therapy or chemotherapy is utilized if aggressive features are noted, but surgery is considered the mainstay for oral cavity cancers.

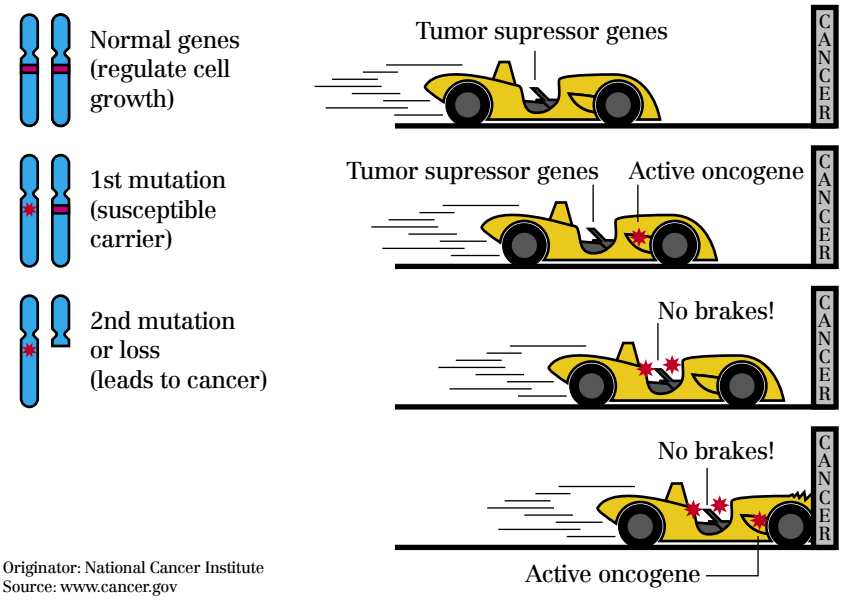
Research into the underlying mechanisms of oral cancer has progressed, and much is known about the DNA alterations that accompany and drive this disease. As underlying mechanisms of carcinogenesis continue to be studied, we discover increasing complexity in the interactions by which a normal cell is transformed into a cancer cell. The general categories of cellular dysfunction involve either activation of oncogenes or disruption of tumor suppressor genes. It is important to

† Johns Hopkins Department of Otolaryngology-Head and Neck Surgery, Milton J. Dance Center for Head and Neck Rehabilitation, Greater Baltimore Medical Center, Baltimore, MD

note these genes have normal cellular functions, but it is only when they are aberrantly turned on or off that the potential for unregulated growth occurs.

This review will discuss the fundamentals of cancer biology and touch on some of the basic DNA changes that are known to occur in OSCC. The molecular techniques used to detect such changes will be highlighted as they have relevance in the possible clinical applications that arise from these discoveries, both from a diagnostic and therapeutic standpoint.

Figure 1: Mutations in tumor suppressor genes



Originator: National Cancer Institute
Source: www.cancer.gov

Both copies of a tumor suppressor gene must be lost or mutated for cancer to occur. A person who carries a germline mutation in a tumor suppressor gene has only one functional copy of the gene in all cells. For this person, loss or mutation of the second copy of the gene in any of these cells can lead to cancer.

The basics: oncogenes and tumor suppressor genes

The link between human genes and carcinogenesis is one that now appears intuitive, but just 30 years ago, this was not the case. Investigators stumbled upon the first proto-oncogene with the hypothesis that virally-mediated infections caused cancer. Their study of the Rous sarcoma virus led to the discovery that the virus did not code for a product that led to carcinogenesis; but rather, it drove the promoter region of a normal avian gene (*src*) which led to the development of cancer.[1] Thus, a proto-oncogene referred to a gene that had the potential to transform a cell into a malignancy, and could exist in the host genome and have a normal cellular function when undisturbed. This finding laid the groundwork for numerous studies looking for the signature alterations in the human genome for different cancers initially associated with retroviruses. However, with the knowledge that these proto-oncogenes existed in their normal state within the human genome, investigators sought to find whether there were other non-viral alterations that could also lead to carcinogenesis.

The field of cancer biology thus expanded to include oncogenes, a class of genes whose normal function is somehow linked to promoting growth and survival of the cell. However, when mutations occur that lead to inappropriate expression of these genes, the normal cell cycle of division and apoptosis (programmed cell death) can be disrupted, and the cell becomes immortal or gains a growth advantage. The prototypical oncogene is *K-ras*, which exerts its effects through a complex cascade of signal transduction. This gene was also first discovered through study of retroviruses,[2] but has proved to be more applicable to human cancers including pancreas, thyroid, lung, and colon. Single point mutations lead to constitutive activation of the *K-ras* gene, which can lead to activation of the mitogen activating protein kinase (MAP-K) pathway. The MAP-K pathway regulates a host of cellular function including mitosis, cell survival, apoptosis, and differentiation.[3]

Tumor suppressor genes define a complementary set of genes whose functions are to inhibit cell growth. Cells are programmed to undergo apoptosis at some point during their lifespan. When the genetic machinery involved in this normal cellular event is abrogated, the cell has the potential to become immortal and escape the apoptotic pathway. The classic tumor suppressor gene is *p53*,[4] which is the most common mutation across all human cancers. Other tumor suppressor genes may have a function less clearly involved in promoting cell proliferation, with possible roles such as DNA repair or cell adhesion. Mutations in these types of genes might lead to a cell being more susceptible to mutagens or having a greater chance of hematogenous or lymphatic metastasis because of weaker cell-to-cell bonding (see Figure 1). Thus, the definition of a tumor suppressor gene can be rather broad.

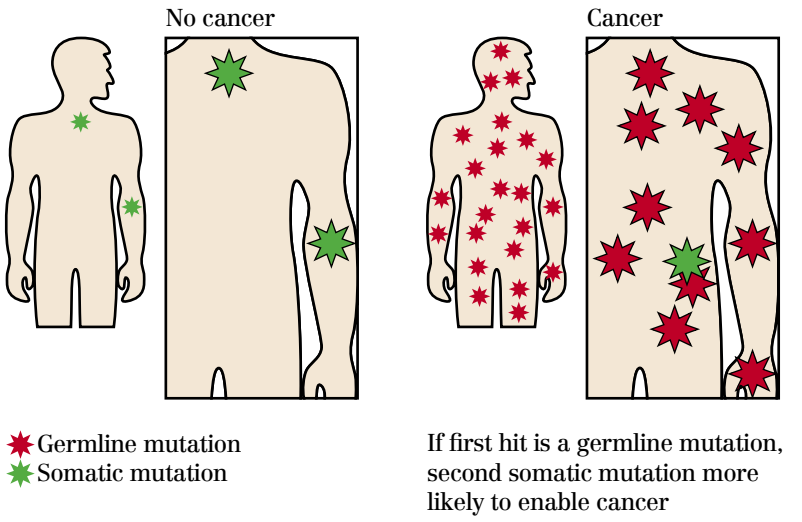
The alterations of these oncogenes and tumor suppressor genes have been detected by molecular assays focused on looking at the DNA sequence. Base pair deletions, insertions, and translocations have all been identified in various tumor models at different sites. There appears to be

characteristic alterations involved in different types of cancers, though solid tumors rarely exhibit a homogenous pattern. We will now examine many of the alterations noted in oral cancer and why these targets may be useful as a means of detection, prognostic indication, and therapeutic intervention.

Knudson's two-hit hypothesis and loss of heterozygosity

In 1975, Knudson created a mathematical model that helped explain why patients with retinoblastoma were more prone to developing subsequent malignancies.[5] Coined the "two-hit" hypothesis (see Figure 2), it describes the increased susceptibility in the development of cancer in those patients who have already inherited 1 defective copy of the *Rb* tumor suppressor gene. Keep in mind there are normally 2 alleles of each gene in humans. Those patients with only 1 functional copy are more likely to acquire a mutation in the remaining normal allele and develop cancer at an early age.

Figure 2: Two-hit hypothesis



Originator: National Cancer Institute
Source: www.cancer.gov

In 1971, Dr. Alfred Knudson proposed the two-hit hypothesis to explain the early onset at multiple sites in the body of an inherited form of cancer called hereditary retinoblastoma. Inheriting one germline copy of a damaged gene present in every cell in the body was not sufficient to enable this cancer to develop. A second hit (or loss) to the good copy in the gene pair could occur somatically, though, producing cancer. This hypothesis predicted that the chances for a germline mutation carrier to get a second somatic mutation at any of the multiple sites in his/her body cells was much greater than the chances for a noncarrier to get two hits in the same cell. Tumor suppressors act recessive at the phenotypic level (both alleles must be mutated/lost for cancer to develop), but the "first hit" germline mutation at the genotypic level is actually inherited in an autosomal dominant fashion.

While the *Rb* gene has not been implicated in OSCC, the principle that both alleles of a tumor suppressor gene must be rendered nonfunctional for cancer to develop has been utilized for molecular detection purposes. Loss of heterozygosity (LOH) (see Figure 3) defines a concept whereby the consistent detection of deletion of 1 allele in a cancer model raises the possibility that the other allele is mutated or altered and might identify a tumor suppressor gene locus. This phenomenon can be assessed by simple polymerase chain reaction (PCR) techniques, and researchers can use LOH as a means of defining novel tumor suppressor genes, or in determining what patterns of loss exist for various tumor types.

Microsatellites and the OSCC progression model

Microsatellite repeats are scattered throughout the genome and represent tandem repeats of noncoding sequences. These can be well detected for sites on various chromosomes and have been mapped to represent surrogate markers for particular sites, which could include tumor suppressor genes. If LOH exists at a site where a known tumor suppressor gene resides, then there is an assumption that the patient is rendered more susceptible to the development of cancer, similar to Knudson's two-hit hypothesis.

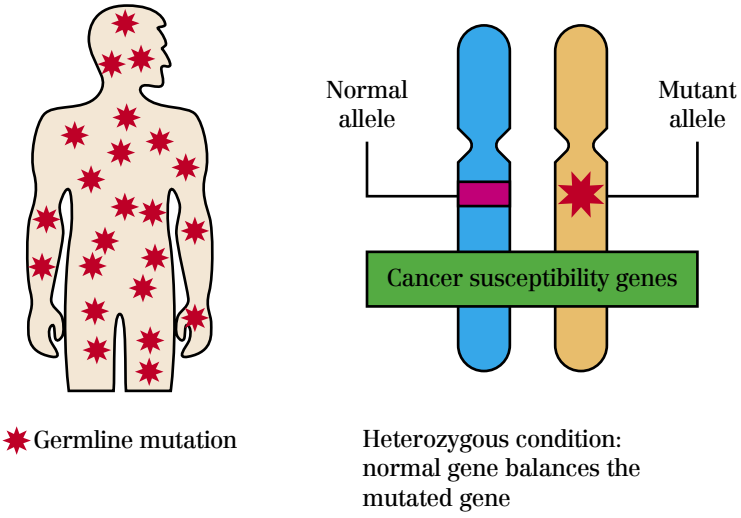
This technique of microsatellite analysis for LOH was performed by Califano and colleagues[6] in oral lesions ranging from benign hyperplasia to invasive cancer, and they discovered there was a characteristic pattern of loss that occurred even in early dysplasias. Thus, a progression model for OSCC was created demonstrating there were detectable DNA alterations in the early benign lesions, and cancer represented an accumulation of these alterations.

This study was meaningful because it was the first demonstration of a linear relationship between the early dysplasias and the frankly invasive carcinomas. Furthermore, it opened the door to the possibility of detection of these genetic alterations for diagnostic, prognostic, and even therapeutic interventions.

Cytogenetics: karyotyping, comparative genomic hybridization, fluorescence based in-situ hybridization

One of the earliest and most common methods of analyzing

Figure 3: Loss of heterozygosity



Originator: National Cancer Institute
Source: www.cancer.gov

In hereditary cancer syndromes, individuals are called heterozygous (having one or more dissimilar gene pairs) because they start life with a germline mutation in one of the alleles linked to cancer susceptibility; however, it is balanced by a normal counterpart. These individuals are predisposed to cancer because all their cells have already sustained the first hit to cancer-linked genes. If the critically needed normal suppressor gene that balances this germline mutation is lost at some time during an individual's life, a condition called loss of heterozygosity (LOH) occurs.

the DNA of individuals and those with cancer was looking at the coiled structure of the chromosomes. Characteristic banding patterns could be seen with some diseases, including leukemias which often involved translocations of large portions of chromosomal arms which could be readily detected. Cytogenetic techniques have advanced such that one can perform whole genome screens looking for amplifications, deletions, or translocations, or one can focus on very specific chromosomal regions using hybridization techniques. While micro-satellites offer the ability to finely map areas of DNA loss, they cannot detect large chromosomal abnormalities, translocations, or amplifications. Thus, the cytogenetic approach offers a different method of detecting areas of DNA alterations that then can be further investigated by more sensitive and focused techniques.

These cytogenetic techniques have been performed in OSCC and have revealed that there are many gross abnormalities in DNA copy numbers and various translocations that occur. In addition, there have been many findings that have pointed to specific chromosomal regions that seem to be hotspots for either amplification or loss.[7-10] Future work will be required to elucidate the gene products that might be responsible for carcinogenesis at these particular sites.

Epigenetic events: promoter hypermethylation

While oncogenes and tumor suppressor genes involve alterations in the DNA sequence, there are other mechanisms controlling the expression of genes, known as epigenetic changes. DNA methylation is one such mechanism. The promoter region of up to 50% of human genes contains cytosine-guanine pairings that exceed the expected frequency just because of chance alone. These areas are known as CpG islands. The 5' carbon of the cytosine is prone to the addition of a methyl group by a series of poorly understood enzymes known as methyltransferases. When this occurs, a binding complex is recruited which blocks the ability of RNA polymerase to transcribe the following gene. It is thought methylation plays a large role in controlling DNA expression during normal development, when genes need to be turned on and off at precise times. Thus, there is no true alteration in the sequence of the gene, but it is silenced by promoter hypermethylation. This means of gene silencing fits in well with Knudson's hypothesis in that 1 allele of a tumor suppressor gene can be deleted or mutated, while another allele is silenced through promoter hypermethylation. Recent studies provide strong evidence that promoter hypermethylation is an important mechanism of tumor suppressor gene silencing in many different tumor models.[11]

In OSCC, there have been many studies detailing the presence of promoter hypermethylation of many known tumor suppressor genes.[12] From a therapeutic standpoint, it is also appealing in that the normal DNA sequence has not been altered, and the methylation is theoretically a reversible process. Future studies are sure to follow in an effort to identify novel tumor suppressor genes that are silenced solely by methylation and to test the feasibility of novel therapeutic agents that are able to target these genes.

Mitochondrial mutations

The role of mitochondria in cellular function has long been known — oxidative phosphorylation occurs in these structures, but they also have a role in carrying out apoptosis and cellular proliferation, and affect the balance of reactive oxygen species within a cell. The body of litera-

ture linking mitochondria to cancer has been growing steadily and is worth mention.

Study of a hotspot of mitochondrial genome mutation known as the D-loop revealed frequent alterations in oral premalignant lesions that increased in incidence with disease severity.[13] While these mutations do not necessarily confer any functional significance, there is at least an association between oral cancer and mitochondrial mutations. Further studies have demonstrated that there is also a progressive increase in mitochondrial DNA content from mild dysplasia to OSCC[14] as well as in the saliva of patients with head and neck cancer.[15] Interestingly, the same investigators found that the salivary mitochondrial DNA content decreased after definitive treatment of these patients, suggesting that it is at least a marker of disease status.[16] More mechanistic studies implicating a direct link between mitochondrial dysfunction and OSCC are emerging,[17,18] and the pathways will become better elucidated in the future.

Clinical applications

There are many possible applications of the molecular findings ranging from detection to therapeutics; however, it is obvious our understanding of the molecular biology and genetics of oral cancer is rudimentary, especially when it comes to targeted therapy. The pathways leading to cancer are complex, and while trends can be determined, there is no alteration that exists in all oral cancers. It is therefore important to accept there will be differences between patients and to tailor the detection or treatment schemes to fit the majority.

Screening/early detection (serum, saliva)

The assays that we have briefly mentioned above used to detect genetic alterations in tumor cells are very sensitive and can be used to find even one cancer cell in a sea of normal cells. Once a DNA abnormality can be clearly characterized in OSCC (such as gene deletion, mutation, or methylation) it can be used as a marker for detection. It is important to note there is not necessarily functional relevance, as long as it is altered in the cancer cells and not in normal cells.

Researchers have used these markers effectively in studies of OSCC, mostly as proof of concept, rather than in clinical trials. The serum or plasma of patients with OSCC contain shed DNA fragments with alterations that match those of the tumor.[19] Microsatellite alterations found in the serum of head and neck cancer patients can even be used to monitor therapy, as these mutations disappeared in successfully treated patients.[20,21] Promoter hypermethylation assays have also been used to detect serum abnormalities in head and neck cancer patients with good success.[22]

Salivary rinses or scrapings have also been used as a more direct means of sampling with the thought that shed tumor cells would be collected along with normal cells. The detection of molecular abnormalities is a very sensitive assay, ideal for this situation, and early studies looking at *p53* mutations in saliva confirmed this notion.[23] Both microsatellite alterations[24] and promoter hypermethylation[25] using salivary rinses have been studied with encouraging results.

The possibilities for these types of assays are exciting, as one could envision performing a simple blood test as a method of screening high risk populations for cancer. Information on the likelihood of cancer development could also be determined in those patients with premalignant disease. Treatment effect could be monitored using blood tests or salivary rinses, and perhaps even the prediction of chemo-sensitivity could be performed by looking at a panel of genes that encompass different molecular alterations and corresponding therapies.

Margin analysis

Another practical application of these molecular diagnostics is in surgical margin analysis. Currently, we rely on histopathologic analysis to determine whether a resection is adequate. Of course, there are situations where local recurrence happens despite an apparently complete excision. This raises the question of whether there were molecular abnormalities in the adjacent tissue that just wasn't yet reflected in their histologic appearance.

In one of the first studies to examine the utility of molecular margin analysis, investigators used *p53* as a detection marker in the surgical margins of patients undergoing resection of head and neck cancers.[26] They discovered 13/25 margins that were called negative by histologic exam were in fact positive for the same *p53* mutation as found in the tumor. Five of those 13 patients with *p53* positive margins developed locoregional recurrence whereas 0/12 of those patients without detectable *p53* at the margins developed recurrence. The use of *p53* or any other marker might prove to be a useful adjunct to routine histologic evaluation, just as immunohistochemical markers have proven to be beneficial in certain situations. A similar study using LOH as a marker for premalignant lesions also demonstrated the utility of including molecular information for margin analysis.[27]

Another intriguing study involved the use of a panel of methylated genes as tumor markers for margin analysis.[28] The interesting aspect of this proof of concept study was that the assay could be performed real time within the context of a longer case requiring free flap closure such that molecular information could be returned in

enough time for further margins to be taken. Thus, these assays can be optimized to give rapid results that could have applications similar to that of frozen section analysis.

Chemoprevention

The notion of being able to prevent or reverse the accumulation of these acquired molecular events is appealing, especially in high risk individuals. There is a high rate of second primary development in patients as they generally have subjected their entire upper aerodigestive tract mucosa to the same environmental exposures. Some of the studies looking into chemopreventive agents follow general classes of compounds such as vitamins and minerals, antioxidants, and molecular targets. It is important to identify molecular pathways that can be targeted with relatively nontoxic compounds that would be safe for routine use as well as have the desired effect.

Retinoids have been the most widely studied compound, and their effects are thought to be because of a re-expression of retinoic acid receptors which are lost in premalignant lesions.[29] Clinical trials have been performed looking into the benefits of high-dose vitamin A in head and neck and lung cancer patients, but there was no apparent effect.[30] As there were concerns about the toxicity profile of high-dose retinoids, a subsequent trial looked into low-dose isotretinoin; however, the results were also disappointing.[31]

COX-2 inhibitors have received attention as there have been studies suggesting that the downstream effects of prostaglandin E2 include activation of EGFR.[32] This association is most strongly seen in patients with colonic polyps and indeed, a clinical trial of patients with familial adenomatous polyposis given COX-2 inhibitors demonstrated a reduction in the number of polyps.[33] There have been animal studies looking at its effects in oral premalignant disease with encouraging results,[34] and clinical trials are ongoing to see its effects in humans.

Gene therapy

The identification of molecular alterations in OSCC is also appealing in that one can replace defective gene products or target cancer cells based on their specific gene alterations. There have been a number of different delivery mechanisms tried, each with varying degrees of success. In OSCC, adenoviral vectors have been used,[35] retrovirus,[36] and even direct injection of plasmid.[37] Other approaches such as the delivery of sensitizing genes through adeno-associated virus has been used with success as well, so called "suicide gene" therapy.[38] The ONYX-015 replication selective adenovirus is able to target *p53* mutant cells and initially had promising results,[39] but further trials have not been continued because of lack of funding. More recently, specific inhibition of gene products in OSCC has been attained using small molecules that interfere with RNA (RNAi).[40,41] These have the benefit of being targeted and relatively easy to deliver. While these do not act on the level of the DNA, their selection is based on the earlier studies implicating various genes and gene products within OSCC. It is beyond the scope of this article to discuss gene therapy in detail, but it is mentioned as an important therapeutic goal and practical application of the molecular data.

Conclusion

We have covered a broad overview of the molecular alterations that accompany oral cancer and precancer. The mechanisms continue to become more numerous and complex, but the understanding of these fundamentals is critical to the development of therapeutic agents as well as molecular diagnostics. Future directions will extend beyond the field of genomics and into gene expression and proteomics, which will also expand the possibilities for targeted therapy.

References

1. Stehelin D, Varmus HE, Bishop JM, et al. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature*. 1976:260:170-173.
2. Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. *Proc Natl Acad Sci U S A*. 1982;79:3637-3640.
3. Pearson G, Robinson F, Gibson TB, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev.* 2001;22:153-183.
4. DeLeo AB, Jay G, Appella E, et al. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc Natl Acad Sci U S A*.1979;76:2420-2424.
5. Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A*. 1971;68:820-823.
6. Califano J, van der Riet P, Westra W, et al. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res*. 1996;56:2488-2492.
7. Liehr T, Ries J, Wolff E, et al. Gain of DNA copy number on chromosomes 3q26-qter and 5p14-pter is a frequent finding in head and neck squamous cell carcinomas. *Int J Mol Med.* 1998;2:173-179.
8. Squire JA, Bayani J, Luk C, et al. Molecular cytogenetic analysis of head and neck squamous cell carcinoma: By comparative genomic hybridization, spectral karyotyping, and expression array analysis. *Head Neck*. 2002;24:874-887.
9. Uchida K, Oga A, Okafuji M, et al. Molecular cytogenetic analysis of oral squamous cell carcinomas by comparative genomic hybridization, spectral karyotyping, and fluorescence in situ

hybridization. *Cancer Genet Cytogenet*. 2006;167:109-116.
10. Singh B, Gogineni SK, Sacks PG, et al. Molecular cytogenetic characterization of head and neck squamous cell carcinoma and refinement of 3q amplification. *Cancer Res*. 2001;61:4506-4513.
11. Esteller M, Corn PG, Baylin SB, et al. A gene hypermethylation profile of human cancer. *Cancer Res*. 2001;61:3225-3229.
12. Ha PK, Califano JA. Promoter methylation and inactivation of tumour-suppressor genes in oral squamous-cell carcinoma. *Lancet Oncol.* 2006;7:77-82.
13. Ha PK, Tong BC, Westra WH, et al. Mitochondrial C-tract alteration in premalignant lesions of the head and neck: a marker for progression and clonal proliferation. *Clin Cancer Res.* 2002;8:2260-2265.
14. Kim MM, Clinger JD, Masayesva BG, et al. Mitochondrial DNA quantity increases with histopathologic grade in premalignant and malignant head and neck lesions. *Clin Cancer Res.* 2004;10:8512-8515.
15. Jiang WW, Masayesva B, Zahurak M, et al. Increased mitochondrial DNA content in saliva associated with head and neck cancer. *Clin Cancer Res.* 2005;11:2486-2491.
16. Jiang WW, Rosenbaum E, Mambo E, et al. Decreased mitochondrial DNA content in posttreatment salivary rinses from head and neck cancer patients. *Clin Cancer Res.* 2006;12:1564-1569.
17. Sasabe E, Tatemoto Y, Li D, et al. Mechanism of HIF-1alpha-dependent suppression of hypoxia-induced apoptosis in squamous cell carcinoma cells. *Cancer Sci.* 2005;96:394-402.
18. Kim MM, Glazer CA, Mambo E, et al. Head and neck cancer cell lines exhibit differential mitochondrial repair deficiency in response to 4NQO. *Oral Oncol.* 2006;2:201-207. Epub 2005 Nov 2002.
19. Nawroz H, Koch W, Anker P, et al. Microsatellite alterations in serum DNA of head and neck cancer patients. *Nat Med.* 1996;2:1035-1037.
20. Nawroz-Danish H, Eisenberger CF, Yoo GH, et al. Microsatellite analysis of serum DNA in patients with head and neck cancer. *Int J Cancer*. 2004;111:96-100.
21. Hamana K, Uzawa K, Ogawara K, et al. Monitoring of circulating tumour-associated DNA as a prognostic tool for oral squamous cell carcinoma. *Br J Cancer*. 2005;92:2181-2184.
22. Sanchez-Cespedes M, Esteller M, Wu L, et al. Gene promoter hypermethylation in tumors and serum of head and neck cancer patients. *Cancer Res*. 2000;60:892-895.
23. Boyle JO, Mao L, Brennan JA, et al. Gene mutations in saliva as molecular markers for head and neck squamous cell carcinomas. *Am J Surg.* 1994;168:429-432.
24. Spafford MF, Koch WM, Reed AL, et al. Detection of head and neck squamous cell carcinoma among exfoliated oral mucosal cells by microsatellite analysis. *Clin Cancer Res.* 2001;7:607-612.
25. Rosas SL, Koch W, da Costa Carvalho MG, et al. Promoter hypermethylation patterns of p16, O6-methylguanine-DNA-methyltransferase, and death-associated protein kinase in tumors and saliva of head and neck cancer patients. *Cancer Res.* 2001;61:939-942.
26. Brennan JA, Mao L, Hruban RH, et al. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N Engl J Med.* 1995;332:429-435.
27. Zhang L, Poh CF, Lam WL, et al. Impact of localized treatment in reducing risk of progression of low-grade oral dysplasia: molecular evidence of incomplete resection. *Oral Oncol*. 2001;37:505-512.
28. Goldenberg D, Harden S, Masayesva BG, et al. Intraoperative molecular margin analysis in head and neck cancer. *Arch Otolaryngol Head Neck Surg.* 2004;130:39-44.
29. Lotan R, Xu XC, Lippman SM, et al. Suppression of retinoic acid receptor-beta in premalignant oral lesions and its up-regulation by isotretinoin. *N Engl J Med.* 1995;332:1405-1410.
30. van Zandwijk N, Dalesio O, Pastorino U, et al. EUROSCAN, a randomized trial of vitamin A and N-acetylcysteine in patients with head and neck cancer or lung cancer. For the European Organization for Research and Treatment of Cancer Head and Neck and Lung Cancer Cooperative Groups. *J Natl Cancer Inst.* 2000;92:977-986.
31. Khuri FR, Lee JJ, Lippman SM, et al. Randomized phase III trial of low-dose isotretinoin for prevention of second primary tumors in stage I and II head and neck cancer patients. *J Natl Cancer Inst.* 2006;98:441-450.
32. Pai R, Soreghan B, Szabo IL, et al. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med.* 2002;8:289-293.
33. Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med.* 2000;342:1946-1952.
34. Feng L, Wang Z. Chemopreventive effect of celecoxib in oral precancers and cancers. *Laryngoscope*. 2006;116:1842-1845.
35. Gibson SA, Pellenz C, Hutchison RE, et al. Induction of apoptosis in oral cancer cells by an anti-bcl-2 ribozyme delivered by an adenovirus vector. *Clin Cancer Res.* 2000;6:213-222.
36. Cardinali M, Jakus J, Shah S, et al. p21(WAF1/Cip1) retards the growth of human squamous cell carcinomas in vivo. *Oral Oncol.* 1998;34:211-218.
37. Harada K, Supriatno, Kawaguchi S, et al. High antitumor activity using intratumoral injection of plasmid DNA with mutant-type p27Kip1 gene following in vivo electroporation. *Oncol Rep.* 2005;13:201-206.
38. Fukui T, Hayashi Y, Kagami H, et al. Suicide gene therapy for human oral squamous cell carcinoma cell lines with adeno-associated virus vector. *Oral Oncol.* 2001;37:211-215.
39. Ries S, Korn WM. ONYX-015: mechanisms of action and clinical potential of a replication-selective adenovirus. *Br J Cancer*. 2002;86:5-11.
40. Yanamoto S, Iwamoto T, Kawasaki G, et al. Silencing of the p53R2 gene by RNA interference inhibits growth and enhances 5-fluorouracil sensitivity of oral cancer cells. *Cancer Lett.* 2005;223:67-76. Epub 2004 Nov 25.

41. Kudo Y, Kitajima S, Ogawa I, et al. Small interfering RNA targeting of S phase kinase-interacting protein 2 inhibits cell growth of oral cancer cells by inhibiting p27 degradation. *Mol Cancer Ther.* 2005;4:471-476.