Focus on head and neck cancer

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is one of the most common types of human cancer, with an annual incidence of more than 500,000 cases worldwide. In the United States alone, approximately 28,000 men and 12,000 women are diagnosed with HNSCC each year, representing 3.2% of all newly diagnosed cancers; the disease also accounts for 2.1% of cancer-related deaths in the United States (Jemal et al., 2004). HNSCC is associated with severe disease- and treatment-related morbidity and has a 5-year survival rate of approximately 50%; this rate has not improved in more than 2 decades (Forastiere et al., 2001).

HNSCC is a complex disease arising in various organs, including the oral cavity, pharynx, and larynx. Tumors from these different sites have distinct clinical presentations and clinical outcomes, and are treated with different strategies, which may include surgery, radiotherapy, and chemotherapy. Recent molecular studies have advanced our understanding of the disease and provided a rationale to develop novel strategies for its early detection, classification, prevention, and treatment.

Tobacco use and alcohol intake are the major risk factors for development of HNSCC (Decker and Goldstein, 1982). However, the exact mechanisms by which tobacco carcinogens and alcohol induce transformation and malignant progression of epithelial cells in the head and neck are not fully understood. The fact that most tobacco and alcohol users do not develop HNSCC in their lifetimes and that approximately 20% of patients with HNSCC, particularly female and those with younger ages, have no clear evidence of exposure to the common risk factors underscores the complexity of HNSCC tumorigenesis and the important role of gene-environment interactions in the tumorigenic process.

Like most epithelial cancers, HNSCC develops through the accumulation of multiple genetic and epigenetic alterations in a multistep process (Figure 1). Deletion of tumor suppressor regions, mutations and de novo promoter methylation of tumor suppressor genes, and amplification or overexpression of oncogenes have been frequently detected in HNSCC. Many of these alterations occur early in tumorigenesis and are identified in premalignant lesions of the head and neck—and even in histologically normal tissues—that are chronically exposed to common carcinogens. Further understanding of the molecular process behind HNSCC tumorigenesis will be essential for the development of novel strategies for early diagnosis and cancer risk assessment, molecular classification, and targeted prevention and treatment (Figure 1).

Etiology

Through a large number of epidemiologic studies, a strong link has been established between the use of tobacco and an increased risk for HNSCC. Alcohol is another independent risk factor for the disease that may have a synergistic effect when combined with tobacco. The risk for development of oral cancer is 3 to 9 times greater in those who smoke or drink and as much as 100 times greater in those who both smoke and drink heavily than in those who neither smoke nor drink (Neville and Day, 2002). Mutations of *p53* have been found more frequently in the HNSCC of smokers and drinkers than in those of other patients (Brennan et al., 1995), suggesting that inactivation of the *p53* tumor suppressor gene is important in tobacco- (and possibly alcohol-) induced HNSCC tumorigenesis.

Human papillomavirus (HPV) may play an important role in the etiology of HNSCC, particularly for tumors of the oropharynx (Gillison et al., 2000). The E6 protein found in the oncogenic high-risk types of HPV, such as HPV-16, can bind to the p53 protein and accelerate its degradation, thereby limiting its ability to inhibit growth of, induce apoptosis in, or cause substantial genetic damage to cancer cells. Thus, it is not surprising that HNSCC with *p53* mutations rarely carry HPV, while oropharyngeal tumors with E6-protein expression lack *p53* mutations (Gillison et al., 2000; Mork et al., 2001), supporting the role of HPV in the development of a subset of HNSCC.

Other risk factors for HNSCC include poor hygiene in the oral cavity; environmental contaminants such as paint fumes, plastic byproducts, and gasoline fumes; gastroesophageal reflux disease; dietary factors; and use of marijuana. In patients with HNSCC who lack clear exposure to the common risk factors, the identification of the etiology of HNSCC will be critical to develop strategies to prevent and treat the disease.

Histologic and clinical classification

HNSCC is graded histologically as well, moderately, or poorly differentiated carcinoma. Well-differentiated tumors contain orderly stratification and heavy keratinization in a pear formation. Moderately differentiated tumors have prickle cells, some stratification, and less keratinization. Poorly differentiated tumors are still recognizable as squamous cell carcinomas but manifest prominent nuclear pleomorphisms and atypical mitosis. However, although the histologic differentiation status is required in pathology reports of HNSCC, it provides limited information to guide treatment decisions because a strong association between differentiation status and clinical outcome or treatment response is lacking.

The anatomic location of HNSCC is important for their clinical classification because tumors arising from different locations often have distinct clinical outcomes and different functional considerations. For example, tumors in the hypopharynx have a higher probability of metastasizing compared to tumors in the oral cavity or larynx. By contrast, laryngeal tumors metastasize infrequently, and patients with these tumors often receive no or minimal surgery but do receive chemoradiotherapy in order to retain their speech function (Forastiere et al., 2003).

The TNM staging system is important in clinical practice to guide treatment selection, and survival of HNSCC patients is

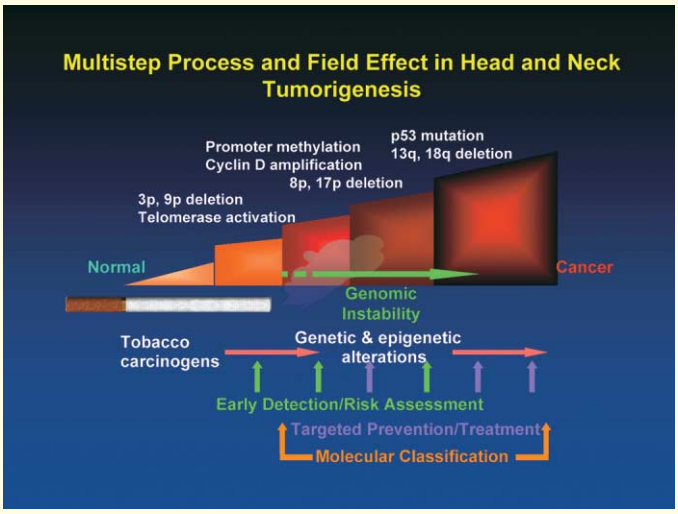

Figure 1. Multistep process and field effect in HNSCC tumorigenesis

Chronic exposure to tobacco carcinogens in the upper aerodigestive tract causes genetic and epigenetic damage in epithelial cells. Accumulation of this damage leads to the development of premalignant lesions and invasive cancers. Understanding the common genetic and epigenetic alterations that are important in the tumorigenic process is critical in the development of molecularly based management strategies.

strongly associated with TNM stage. The system is based on tumor size and invasion features (T stage), regional lymph node spread (N stage), and the presence of distant metastasis (M stage). However, patients classified as having the same stage of disease may differ substantially in their response to the same treatment and in their clinical outcome, indicating the need for a staging system based on molecular characteristics of the tumors.

Molecular basis of HNSCC tumorigenesis

Deletion of one of the two alleles at chromosomes 3p (multiple regions) and 9p21 is the most frequent event in lesions of the head and neck with only mild histologic changes, such as hyperplasia and mild dysplasia, and even in some normal-appearing epithelial cells (Mao et al., 1996a). Because these chromosomal regions harbor tumor suppressor genes, such as *FHIT* at 3p14 and $p16^{INK4a}$ at 9p21, such deletions might affect malignant transformation. This theory is supported by the observation that the lesions of oral leukoplakia with deletions at 3p14 and 9p21 carry a higher risk for transformation into invasive tumors (Mao et al., 1996a; Rosin et al., 2000). Oral leukoplakia is the most common head and neck premalignant lesion and has a malignant transformation rate as high as 24% (Papadimitrakopoulou et al., 1997). However, our ability to predict the malignant potential of these lesions based on pathologic findings is very limited. Given the identification of multiple molecular alterations in these lesions, molecular markers may have promise in improving our ability to determine the true cancer risk of these lesions. Another important event in the early tumorigenic process of HNSCC is the reactivation of telomerase (Mao et al., 1996b; Mutirangura et al., 1996) prolonging survival of cells with genetic abnormalities and making accumulation of multiple genetic alterations possible. Califano et al. analyzed head and neck lesions at various stages of tumorigenesis to detect deletions at multiple chromosomal regions and proposed a progression model (Califano et al., 1996).

The *p53* tumor suppressor gene is the most frequently mutated gene in HNSCC, with a frequency close to 50% (Brennan et al., 1995). Inactivation of *p53* makes HNSCC more resistant to radiotherapy and oral premalignancies less sensitive to retinoid-based chemopreventive strategies (Koch et al., 1996; Shin et al., 2000). Gene amplification and protein overexpression have been found in HNSCC. The gene *cyclin D1*, for example, is frequently amplified and overexpressed in early stage head and neck tumorigenesis (Izzo et al., 2003). Overexpression of epidermal growth factor receptor (EGFR) is an important feature in HNSCC and has been the basis for targeting EGFR as a treatment strategy (Pomerantz and Grandis, 2003). *p63*, a *p53* homolog with oncogenic property, is also frequently amplified and overexpressed in HNSCC (Patturajan et al., 2002).

Silencing gene expression by hypermethylation of CpG-rich promoters is a common mechanism to inactivate tumor suppressor genes in many types of cancers, such as *p16*, *DAP-kinase*, and *E-cadherin* in HNSCC (Herman and Baylin, 2003; Hasegawa et al., 2002). Aberrant pre-mRNA splicing is another epigenetic mechanism that may inactivate tumor suppressor genes and generate oncogenes (Bartel et al., 2002). We have shown that this may be an important mechanism to inactivate *FHIT* in HNSCC (Mao et al., 1996c).

Many of these molecular abnormalities, particularly DNA deletions and amplifications, are the consequence of genomic instability. Recent studies have shown that genomic instability (aneuploidy) is critical in oral cancer development (Sudbo et al., 2001) and that patients whose tumors developed from aneuploidy lesions had extremely poor clinical outcomes (Sudbo et al., 2004). Understanding the mechanism for genomic instability may lead to novel preventive or therapeutic strategies preventing or minimizing genomic instability.

Field cancerization and development of multiple tumors

The concept of field cancerization was introduced to explain how multiple primary tumors develop in the upper aerodigestive tract, based on the fact that the entire tract is chronically exposed to common carcinogens, such as tobacco smoke (Slaughter et al., 1953). The concept has been challenged because some clinically diagnosed second primary tumors distant from the original tumor appeared to derive from clonal spreading of the original one based on genetic analysis (Bedi et al., 1996). In a recent study, we showed that clonal spreading occurred in invasive and noninvasive lesions, although the probability of clonal spreading increased with tumor progression. However, we also found that other multiple oral lesions developed independently, supporting a strong role for field cancerization (Jang et al., 2001). Figure 2 shows the location of a patient's multiple oral lesions that developed over a 10-year period to illustrate the effect of carcinogen-induced field cancerization and clonal spreading based on molecular analysis.

Prevention

The development of HNSCC requires accumulation of genetic and epigenetic alterations, and thus can theoretically be interrupted, delayed, or reversed through the use of natural or synthetic agents in a process known as chemoprevention (Hong and Sporn, 1997).

High-dose retinoids have established efficacy in the reversal of early oral premalignant lesions but are associated with mucocutaneous toxicity (Hong et al., 1986). The efficacy of

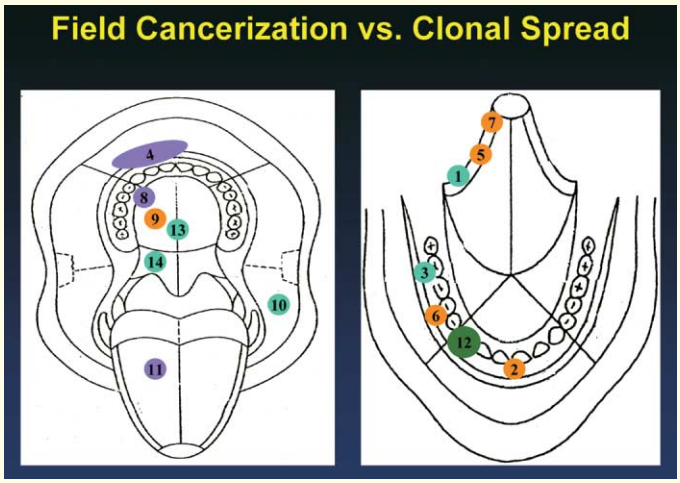

Figure 2. Field cancerization versus clonal spread

The diagram shows the sites of 14 oral lesions that developed in a patient over 10 years. There were nine invasive cancers and five premalignant lesions. Each lesion was treated by complete resection at the time of diagnosis. Each lesion is numbered according to the sequence of diagnosis. Lesions with the same color shared identical genetic alterations, suggesting the same clonal origin based on results of genetic analysis and a mathematic model. Four independent clones might have been responsible for the development of all 14 oral lesions in this patient.

retinoids on moderately and severely dysplastic lesions were addressed with a combination of interferon-α, α-tocopherol, and 13-*cis*-retinoic acid (Papadimitrakopoulou et al., 1999). The study revealed the activity of this combination in reversing histology of laryngeal lesions but with persistence of genetic abnormalities in the tissue despite histologic response (Mao et al., 1998). A phase II trial showed that the same combination of drugs was highly effective as bioadjuvant therapy in patients previously treated for a locally advanced head and neck cancer (Shin et al., 2001).

The possibility of preventing second primary tumors was suggested by a randomized, double-blinded, placebo-controlled trial of high-dose 13-*cis*-retinoic acid taken for 1 year by patients whose original HNSCC were curatively treated (Hong et al., 1990). However, these results were not confirmed by studies with low-dose 13-cis-retinoic acid (Khuri et al., 2003) or with different retinoids (van Zandwijk et al., 2000).

New strategies in chemoprevention include targeting *p53* abnormalities, selective cyclooxygenase-2 inhibitors, EGFR kinase inhibitors, and the epigallocatechin gallate component of green tea. Newer studies should be designed to treat lesions with a higher genetically defined risk for cancer development, such as aneuploidy or loss of heterozygosity at specific tumor suppressor loci in oral premalignant lesions (Sudbo et al., 2001; Mao et al., 1996b; Partridge et al., 2000; Rosin et al., 2000) or patients with certain *cyclin D1* polymorphisms (Izzo et al., 2003).

Current treatment of head and neck cancer

Advances have occurred in all major treatments for HNSCC, including surgery, radiotherapy, chemotherapy, and use of targeted therapeutic agents. The focus of surgical innovation during the past decade has been the conservation of organ function and more effective means of reconstruction. Advances in radiotherapy approaches have involved dividing the dose of radiotherapy to increase its efficacy, including hyperfractionation and accelerated fractionation. Phase III trials testing both of these approaches have documented that they result in a 10% to 15% improvement in local and regional tumor control in patients with middle-stage to advanced-stage disease, although without a significant change in the overall survival rate (Fu et al., 2000; Horiot et al., 1997).

In the past several years, new treatment strategies have incorporated chemotherapy as an integral component of potentially curative therapy aimed at organ preservation, optimal disease control, and prolongation of survival time.

In the late 1980s to mid-1990s, induction chemotherapy followed by radiotherapy achieved the goal of laryngeal preservation, as demonstrated by the Veterans Administration Laryngeal Cancer Study and the similarly designed trial of the European Organization for Research and Treatment of Cancer, for hypopharyngeal cancer, with equivalent survival for patients treated with induction chemotherapy followed by radiotherapy to that of patients treated with standard surgical resection followed by postoperative radiation (Department of Veterans Affairs Laryngeal Cancer Study Group, 1991; Lefebvre et al., 1996).

Over the past 10 years, the simultaneous administration of chemotherapy and radiotherapy has become the subject of intense clinical research. The rationale for this approach lies in its potential to overcome radioresistance within the irradiated field, to eradicate micrometastatic disease, to target different cell subpopulations within the tumor, to enable chemotherapy-associated inhibition of repair of DNA damage induced by radiation, and to cause cell cycle redistribution toward more radiosensitive phases.

A meta-analysis that included 63 randomized studies conducted between 1965 and 1993 showed an absolute survival benefit of 4% at 5 years with the addition of chemotherapy to locoregional treatment. The survival benefit was confined to trials that compared concomitant chemoradiotherapy with radiotherapy alone (absolute survival benefit for chemoradiotherapy, 8% at 5 years) (Pignon et al., 2000). Recent phase III studies have almost uniformly supported the superiority of concurrent chemoradiotherapy over radiation alone in the setting of unresectable HNSCC (Forastiere et al., 2001), in terms of either survival (Adelstein et al., 2003; Jeremic et al., 2000) or organ preservation (Forastiere et al., 2003).

The potential advantages of using multiagent chemoradiotherapy have been investigated in several phase II or III trials. Satisfactory results have been reported in phase II trials with the combination of radiation, 5-fluorouracil, and hydroxyurea (Kies et al., 2001; Vokes et al., 2000). A recent phase II randomized study was conducted by the Radiation Therapy Oncology Group (RTOG 9703) to compare three doublet chemotherapy regimens (cisplatin and 5-fluorouracil, hydroxyurea and 5-fluorouracil, and cisplatin and paclitaxel) administered with conventional radiotherapy (Garden et al., 2001). Although no statistically significant difference in survival rate was shown among three arms, all had superior survival rates compared with historical radiation-alone data from the RTOG. Further randomized studies are needed to establish the optimal combination regimen for chemoradiotherapy programs.

Based on these data, the standard nonsurgical therapy for patients with HNSCC should involve combined radiation and chemotherapy, given concurrently. The optimal chemotherapy regimen and fractionation scheme and the role of induction and adjuvant chemotherapy still remain to be defined.

Given the locoregional pattern of treatment failure in

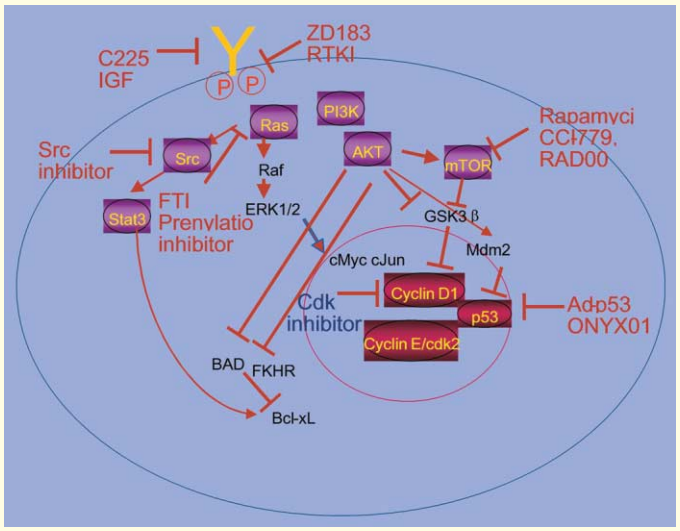

Figure 3. Simplified scheme of the molecular abnormalities within signal transduction pathways in HNSCC that are possible targets for intervention with targeted therapies

C225, cetuximab; IGF, insulin-like growth factor; Abs, antibodies; RTKIs, receptor tyrosine kinase inhibitors; FTI, farnesyl transferase inhibitors; Cdk, cyclin-dependent kinase.

HNSCC and the ominous prognosis for patients with HNSCC who are not candidates for surgery (Vokes et al., 1993), the use of concomitant reirradiation and chemotherapy in such patients is currently under investigation by multiple clinical trial groups, with encouraging preliminary data, especially following surgical debulking (De Crevoisier et al., 2001).

In the setting of recurrent and metastatic disease, single agents with activity in the range of 15% to 30% include methotrexate, cisplatin, carboplatin, 5-fluorouracil, paclitaxel, and docetaxel. Combination chemotherapy has been shown to result in significantly higher response rates than those achieved by single-agent chemotherapy. The combination of cisplatin and 5-fluorouracil given by continuous intravenous infusion is the most frequently used regimen (Forastiere et al., 2001), although it has resulted in no improvement in the median duration of survival of 6 months or in the overall survival rates. Similarly, randomized trials directly comparing newer regimens, such as paclitaxel or docetaxel plus cisplatin or carboplatin, with the standard cisplatin and 5-fluorouracil regimen have not shown superiority in terms of survival rate.

Targeted therapies for head and neck cancer

Development of new agents to treat HNSCC has mainly concentrated on recurrent and metastatic disease, for which no effective chemotherapy is currently available. These new drugs, designed to target specific molecular defects unique to cancer cells (Figure 3), include EGFR inhibitors, tyrosine kinase inhibitors, protein farnesylation inhibitors, and *p53*-replacing therapy.

EGFR overexpression occurs in head and neck premalignant and malignant lesions with a frequency of 80% to 100% and is generally associated with an adverse outcome (Grandis et al., 1998). EGFR is a member of the ErbB receptor tyrosine kinase family; its ligands epidermal growth factor and transforming growth factor-α cause receptor dimerization, and activation of receptor tyrosine kinase and phosphorylation of several substrates result in activation of signal transduction pathways (e.g., the *ras*/mitogen-activated protein kinase, phosphatidylinositol-3 kinase, and signal transducer and activator of transcription-3 pathways) that can lead to cell proliferation, tumor growth, and progression of invasion and metastasis (Salomon et al., 1995).

Many strategies to block or downregulate the EGFR have been developed, including the use of tyrosine kinase inhibitors, monoclonal antibodies, ligand-linked toxins, and antisense oligonucleotides (Mendelsohn and Baselga, 2003). The cellular effects of EGFR blockade include cell cycle arrest, potentiation of apoptosis, inhibition of angiogenesis, and inhibition of tumor-cell invasion and metastasis. Enhanced cytotoxicity has been observed when the monoclonal antibody against the EGFR, cetuximab, is used in combination with a number of conventional cytotoxic therapies plus radiotherapy; this combination works by inhibiting tumor cell repopulation. Cetuximab has also been shown to cause response in 10%–14% of platinum-resistant HNSCC when the antibody was added to a regimen of cisplatin or carboplatin after documented progression of disease (Mendelsohn and Baselga, 2003). However, a randomized clinical trial comparing cisplatin in combination with cetuximab or placebo revealed no statistically significant difference in terms of progression-free survival, despite a higher response rate in the group receiving cetuximab. A phase III randomized trial attempting to confirm the effectiveness of cetuximab in combination with radiotherapy is being evaluated (Mendelsohn and Baselga, 2003).

Reversible and irreversible small molecule tyrosine kinase inhibitors have shown preclinical activity with good bioavailability and have moved into clinical trials, both alone and in combination with chemotherapy. ZD1839 (Iressa, or gefitinib) and OSI-774 (Tarceva, or erlotinib) are reversible tyrosine kinase inhibitors that have demonstrated promising antitumor activity in early clinical trials conducted mainly in patients with advanced non-small cell lung cancer. Major side effects include mild acne-like rash, diarrhea, and nausea. Phase II studies of both compounds have been completed in patients with recurrent and metastatic HNSCC, resulting in response rates of 10% for gefitinib and 4% for erlotinib (Cohen et al., 2003; Soulieres et al., 2004). Ongoing studies in HNSCC are exploring combinations of oral tyrosine kinase inhibitors with radiotherapy and cisplatin.

Approximately 90% of human neoplasms have abnormalities in some component critical for the G1 to S phase cell cycle transition. In HNSCC, cyclin D1 is commonly overexpressed, and p16, the endogenous inhibitor of cyclin-dependent kinase-4, is commonly deleted or not transcribed. Flavopiridol is a pharmacologically potent cyclin-dependent kinase inhibitor that depletes *cyclin D1* and vascular endothelial growth factor mRNA, induces apoptosis, and suppresses the growth of HNSCC. This drug has been tested in clinical trials in patients with HNSCC (Patel et al., 1998); the optimal schedule and combination with standard cytotoxic agents are still being investigated.

The initial premise behind developing farnesyl transferase inhibitors was to inhibit a critical enzymatic activity for the constitutive function of mutated Ras (Lebowitz et al., 1995). However, in preclinical and clinical settings, these compounds appeared to inhibit tumor growth irrespective of their Ras status. A phase IB trial of the farnesyl transferase inhibitor SCH66336 in HNSCC has observed substantial reductions in tumor size, despite short exposure to the drug. A phase II monotherapy trial in recurrent, metastatic HNSCC is ongoing.

Replication-competent adenoviruses that selectively repli-

cate in and cause lysis of cells deficient in *p53* tumor suppressor activity have been developed. Phase I and II clinical trials of the intralesional injection of ONYX-015 in patients with recurrent or refractory HNSCC suggest that the adenovirus is effective and that the responses at injected tumor sites are durable (Khuri et al., 2000). Replacement of the *p53* gene using an adenovirus containing the wild-type *p53* gene (Ad-*p53* or RPR-INGN-201) has been evaluated in a phase I trial in patients with advanced, recurrent HNSCC and has led to some durable responses and to a pathologic complete response (Clayman et al., 1998). Based on these promising initial data, two ongoing trials are testing the combination of Ad-*p53*, cisplatin, and 5-fluorouracil versus cisplatin and 5-fluorouracil alone and testing Ad-*p53* versus methotrexate as a second-line therapy in patients with recurrent or metastatic HNSCC.

Some additional targets worthy of exploration in HNSCC for which either small molecules or antibodies are in clinical development are the mTOR inhibitors, insulin-like growth factor-signaling blockers, histone deacetylase inhibitors, and heat shock protein 90 inhibitors.

Conclusions

Progress in treatment of HNSCC has been slow and challenging. However, a number of studies have established the feasibility and efficacy of combining treatment modalities to enable organ preservation and have laid the framework for circumvention of the treatment plateau encountered with conventional cytotoxic agents through the integration of molecularly targeted agents. The challenges that lie ahead include determining the optimal dose and sequence of these agents, the choice of the appropriate setting for using biologically active agents targeting actions different pathways of tumor growth, and, most important, the timely integration of laboratory-based studies and molecular imaging into the development of new treatments.

Acknowledgments

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