Review

Therapeutic Exploitation of the Physiological and Molecular Genetic Alterations in Head and Neck Cancer

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Abstract

Despite improvements in the diagnosis and management of head and neck squamous cell carcinomas, there has been minimal increase in the long-term survival in these patients over the last 30 years. Treatment intensification with concurrent chemoradiotherapy has been shown to increase survival and improve organ preservation over radiotherapy alone in patients with locally advanced tumor; however, at a cost of increased long-term toxicity. Recent advances in molecular technology have ushered in a new age of targeted therapy, which holds promise for a better outcome for these patients with potentially less normal tissue toxicity. Some of the new approaches aim to specifically inhibit tumor growth and metastasis by targeting the tumor suppressor gene or protein and signal transduction pathways. This review will summarize these new molecular and physiological based strategies that can be used for both treatment and chemoprevention of head and neck squamous cell carcinoma.

Clinical, Pathological, and Molecular Progression of HNSCC²

HNSCC is thought to proceed in an orderly progression from benign squamous hyperplasia to dysplasia to carcinoma in situ to invasive carcinomas. Fig. 1 shows the proposed model of HNSCC progression in the literature (1, 2). Several studies have shown that despite normal appearance, some mucosa may have already harbored early genetic changes, which may be used to screen for patients at risk for tumor progression (3).

Table 1 shows the most frequent molecular alterations in HNSCC (4). Loss of chromosome 9p21 is the most common change and can occur early in tumor progression. p16 is one of the genes found in this region and codes for CDKN2A, an inhibitor of CDK (5). Several additional CDK inhibitors map to band 9p21, including p15/CDKN2B, p18/CDKN2C, and p19/CDKN2D (6). The CDKN2A-D inhibitors block cell cycle progression beyond the G1 checkpoint in eukaryotes by complexing with CDK4 and CDK6, thereby preventing CDK binding to D-type cyclins, and, thus, inhibiting phosphorylation of retinoblastoma protein. Inactivation of CDKN2A-D would lead to loss of cell cycle checkpoint control with subsequent deregulation of cellular proliferation.

Another common chromosomal loss in HNSCC is in the 3p region. 3p loss has been observed in dysplastic oral lesions, suggesting its involvement in early tumor progression (7). Alteration of the FHIT tumor suppressor gene or protein has been observed in HNSCC precursor lesions and invasive carcinomas (8), suggesting that loss of FHIT function may be important in the development of HNSCC. However, additional studies will be required to determine how FHIT affects HNSCC development.

Loss of p53 function via mutations in the gene locus at 17p13 is has been observed in more than half of all HNSCCs. The incidence of p53 mutation increases with progression from preinvasive to invasive lesions and may contribute to tumor aggressiveness (9).

Gain of the long arm of chromosome 3 is another common genetic change. The AIP gene (also known as p40/p73L), which maps to distal 3q, has been observed to have copy number gain and overexpression at both the message and protein levels in HNSCC. Its overexpression in rat 1a cells leads to a transformed phenotype, suggesting its involvement in tumor carcinogenesis and progression (10).

Amplification of 11q13, which harbors the loci of cyclin D1 and cortactin (EMS1) genes, is observed in about 30–50% of HNSCC, and is generally associated with invasive carcinomas (11, 12). Cyclin D1 is a critical cell cycle regulatory protein that drives the cell cycle from G1 to S phase by binding to CDK4 or CDK6, phosphorylating and inactivating retinoblastoma protein. In HNSCC, cyclin D1 overexpression has been associated with higher stage, early nodal spread, poorer response to therapy, and reduced survival (11, 13). Cortactin is an actin binding protein that is involved in the organization of cytoskeleton and cell adhesion structures. Overexpression of cortactin has been noted in 31% of HNSCC tumors (14). Cortactin or EMS1 gene amplification correlates with advanced tumor and nodal stage, poor histological differentiation, and increased risk of tumor recurrence and tumor-related death (14, 15). Increased cortactin protein expression by hypoxia was observed recently in HNSCC variants.
suggesting a link among cortactin, the tumor microenvironment, and tumor progression.

Physiological and Molecular Targets in HNSCC

Targeting the Tumor Microenvironment. Substantial evidence has accumulated to suggest that tumor hypoxia adversely impacts treatment outcomes in HNSCC patients by increasing tumor radioresistance and enhancing tumor progression toward a more malignant phenotype (16, 17). Since the 1950s, enormous efforts have been devoted to develop strategies to overcome the radioresistance of hypoxic cells. A new promising strategy to exploit tumor hypoxia is through the bioreductive release of diffusible cytotoxins, the prototype of which is TPZ (or SR4233). TPZ is a benzotriazine with selective cytotoxicity for hypoxic cells. Mechanistically it has been demonstrated that TPZ undergoes an one-electron reduction to form a cytotoxic free radical that causes DNA breaks, chromosomal aberrations, and cell death (18). In the presence of oxygen, the TPZ radical is back-oxidized to the nontoxic parent compound. Whereas TPZ was first proposed to be used with radiotherapy, recent emphasis has been on the ability of TPZ to potentiate the cytotoxicity of cisplatin. Early results from Phase I-II clinical trials of TPZ in HNSCC patients are encouraging (19). However, a small Phase II randomized study from our institution...

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Table 1 Molecular alteration in head and neck cancers

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Alteration</th>
<th>Frequency (%)</th>
<th>Associated genes</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>3q11-qter</td>
<td>Gain</td>
<td>37–71</td>
<td>AIS (p40/73)</td>
<td>Carcinogenesis, transformed phenotype</td>
</tr>
<tr>
<td>7p12-p22</td>
<td>Gain</td>
<td>34–47</td>
<td>EGFR</td>
<td>Growth and aggressive phenotype</td>
</tr>
<tr>
<td>8q13-q24.3</td>
<td>Gain</td>
<td>27–50</td>
<td>MYC, PTK2</td>
<td>Adhesion and growth regulation</td>
</tr>
<tr>
<td>11q13 (CIS)</td>
<td>Gain</td>
<td>39–61</td>
<td>Cyclin D1, EMS, FGF3, FGF4</td>
<td>Cell cycle progression, migration, growth regulation</td>
</tr>
<tr>
<td>20q12-q13.2</td>
<td>Gain</td>
<td>33–48</td>
<td>BCAS1, ZNF217</td>
<td>High grade, aneuploidy</td>
</tr>
<tr>
<td>3p12-p24 (Dysplasia)</td>
<td>Loss</td>
<td>53–72</td>
<td>FHT</td>
<td>Tumor suppression</td>
</tr>
<tr>
<td>8pter-q21</td>
<td>Loss</td>
<td>62–63</td>
<td>Unknown</td>
<td>? tumor suppression</td>
</tr>
<tr>
<td>9p21-p24 (Hyperplasia)</td>
<td>Loss</td>
<td>39–67</td>
<td>p16, p15, p18, p19</td>
<td>Cell cycle progression, senescence</td>
</tr>
<tr>
<td>18q</td>
<td>Loss</td>
<td>58–59</td>
<td>DCC, DPC4, MADR2, ovalbumin serpin genes, maspin (P15), SCCA1, SCCA2, PAI2, headpin (P15)</td>
<td>Poor prognosis</td>
</tr>
<tr>
<td>17p13</td>
<td>Loss or mutated</td>
<td>55</td>
<td>p53</td>
<td>Cell cycle regulation, apoptosis</td>
</tr>
</tbody>
</table>

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**Fig. 1** A proposed model of progression in HNSCCs (adapted and modified from Refs. 1, 2). The orderly pathological progression is accompanied by chromosomal and genetic changes, many of which occur early in the progression (Courtesy of Dr. Christina Kong, Department of Pathology, Stanford University).

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3 Q-T. Le et al., unpublished observations.
comparing aggressive chemoradiotherapy to the same regimen with TPZ showed no difference in outcomes to date (20). Therefore, a large multi-institutional Phase III trial is being mounted to study the efficacy of TPZ in combination with radiotherapy and cisplatin in HNSCC.4

Another promising approach to overcome tumor hypoxia in HNSCC is the use of the nicotinamide vasodilator and carbogen breathing (ARCON) to increase the oxygen partial pressure of tumors. ARCON in combination with accelerated radiotherapy has produced a >80% 3-year local control rate for advanced stage T3-4 laryngeal and oropharyngeal cancers (21). Presently, a Phase III clinical trial testing the efficacy of ARCON in combination of accelerated radiotherapy in laryngeal cancers is ongoing in Europe with a projected accrual of 344 patients (21). The results of this study will elucidate the role of ARCON in the management of some head and neck cancers.

**Targeting the EGFRs.** Because EGFR is ubiquitously expressed in HNSCC and its expression or overexpression is associated with a poor prognosis (22–24), it is logical that different EGFR blockers are being investigated in HNSCC. These include anti-EGFR monoclonal antibodies, tyrosine kinase small molecule inhibitors, ligand conjugates, immunonjugates, antisense oligonucleotides, and truncated dominant-negative mutant EGFR. Preclinical xenograft studies have shown promising results with evidence of synergism between EGFR blockade and cytotoxic chemotherapy or radiotherapy (25–28). Phase I-II clinical trials have shown that the toxicity of EGFR blockade is mild and confined predominantly to skin rash, gastrointestinal symptoms for tyrosine kinase inhibitors, and allergic reaction for the monoclonal antibodies (IMC 225; Imclone System; Ref. 29). These studies have also shown that EGFR blockade may have encouraging activity in primary HNSCC tumors when combined with radiation therapy and in cisplatin resistant tumors when combined with chemotherapy as suggested by the high tumor response rate (29–31). However, the increased response of tumors with the addition of EGFR blockade to cytotoxic chemotherapy has not translated in improved survival in patients with recurrent HNSCC (IMC-225) or in patients with advanced stage non-small cell lung cancers (ZD1839 or Iressa; AstraZeneca Pharmaceutical; Ref. 32). A Phase III study comparing the combination of IMC-225 and radiotherapy to radiotherapy alone in patients with locally advanced HNSCC has been completed and analysis is under way.5 The discrepancy between impressive preclinical results and the less impressive clinical data suggests that a single EGFR blocking agent alone is insufficient to improve tumor control, or tumor cells can up-regulate downstream pathways to compensate for EGFR blockade. Inadequate blockade of the EGFR pathway can theoretically be overcome by combining different anti-EGFR agents with different mechanisms of action such as IMC225 and Iressa in the same treatment regimens. Potential compensatory up-regulation of downstream pathways can be counteracted with combinations of inhibitors targeting both the EGFR and critical downstream molecules such as Stat-3, Raf, Ras, or mitogen-activated protein kinase. Clinical trials using combinations of targeted therapy in addition to conventional treatment may be necessary to overcome treatment resistance in HNSCC. Another possible explanation for the negative clinical data observed to date is our inability to select the right patient population for EGFR targeting. For example, it may be more efficacious to combine EGFR blockade with traditional therapy in patients with earlier stage tumors or treatment-naïve tumors than in patients with refractory or very advanced disease. Innovative biomarkers to identify patients who will benefit from such an approach are desperately needed to optimize the utility of these compounds.

**Targeting the Vasculature.** A functional vascular network is crucial for the survival and growth of solid tumors, making tumor vasculature a key target for solid tumors, including HNSCC. Tumor angiogenesis manifested as increased VEGF expression or microvessel density counts has been shown to adversely impact treatment results in HNSCC patients in several series (33–39). Most of the work in this area has focused on the development of antiangiogenesis agents, which include antiangiogenic drugs such as thalidomide, peptide inhibitors of endothelial cell proliferation such as angiotatin or endostatin (40, 41), or VEGF inhibitors such as neutralizing antibodies or dominant-negative soluble receptors (42–45). Although preclinical studies showed that inhibition of angiogenesis can block the growth of primary and metastatic experimental tumors, reduce tumor vascularity, and increase apoptosis (42–45), clinical results have been mixed. A Phase II study of thalidomide in recurrent or metastatic HNSCC has yielded disappointing outcomes with no obvious single agent antitumor activity, and no changes in serum VEGF or fibroblast growth factor levels in a group of heavily pretreated patients (46). Phase I dose escalation studies with endostatin in patients with refractory solid tumors showed no significant drug related toxicity, but at the same time also no significant response (47–49). In addition, serial measurements of presumed surrogate markers of angiogenesis such as serum VEGF or fibroblast growth factor levels, positron emission tomography imaging of tumor blood flow or metabolism, or measurement of tumor and endothelial cell apoptosis did not consistently correlate with blood levels of endostatin or treatment response (50, 51). To date, minimal data are available on clinical trials targeting VEGF or its receptors in HNSCC; however, Phase I studies are under way, and their data are emerging.

An alternative to antiangiogenic approach is to target the existing tumor vasculature. Differences in the physiology of immature tumor versus the mature normal vasculature provide an opportunity for the selective disruption of tumor blood flow, leading to tumor death. A potential advantage of this approach is the targeting of central areas of a tumor that can be resistant to conventional therapy. Two types of vascular targeting agents are being developed currently for cancer treatment: the ligand-directed VTAs, which use antibodies and peptides to target tumor endothelium, and the small molecules that can induced selective occlusion of tumor blood vessels. Examples of ligand-indirect compounds include tissue factor antibodies, VEGF fused with plant toxin gelonin, DNA encoding FIK-1 fused to FAS, and so forth. Small molecules VTAs include tubulin-binding agents such as combrestatatin A-4 and ZD 6126, and

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4 E. Loh and S. Synthelabo, personal communication.

5 J. Bonner and P. Harari, personal communication.
flavonoids such as flavone acetic acid and 5,6-dimethylxanthone-4-acetic acid (52–57). A comprehensive review on the current status of VTA was published recently (58). Improved antitumor effects have been observed in preclinical studies when VTAs are combined with other therapy that kill the viable rim of tumor cells that can repopulate tumors (58–60). A number of small molecules VTA such as combrestatin A-4, ZD 6126, and 5,6-dimethylxanthone-4-acetic acid are being tested in Phase I or II clinical trials.

**Targeting RAS.** Up to 27% of oral cavity cancers have mutations in the Ras oncogene (61). FTIs are a class of compounds that inhibit a critical enzymatic step in the constitutive expression of mutated Ras genes. FTI appears to be effective in blocking the growth of a wide variety of cell lines, including HNSCC cell lines and synergistic activity with radiotherapy (62), and conventional chemotherapy such as taxane compounds (63). Several Phase I studies of FTI in combination with chemotherapy have been performed in solid tumors. In operable HNSCC patients, FTI inhibitor SCH66336 (Schering Plough) has been tested preoperatively to obtain pharmacodynamic data, which shows inhibition of DNA-J (a heat shock protein) and laminin-A farnesylation. In addition, some degree of tumor regression was observed in 4 patients receiving the compound (64). FTI-778,123 compound (Merck Research Laboratory) was also tested in combination with radiotherapy in a Phase I study that included 3 HNSCC patients. No enhanced toxicity was observed (65). Efficacy data are not yet available. Preclinical studies suggest that FTIs increase tumor oxygenation, which may result in increased efficacy of radiotherapy (66).

One downstream target of Ras is PI3K, which phosphorylates AKT. Elevated phosphorylated AKT staining in archival HNSCC tissues has been shown to confer a poor local control rate in HNSCC patients treated with chemoradiotherapy (62). Pharmacological inhibition of PI3K can lead to similar levels of tumor radiosensitization to those found with inhibition of Ras. Thus, inhibition of Ras can lead to increased tumor control by radiotherapy by both increasing tumor oxygenation and inhibiting PI3K activity.

**Targeting p53.** Because p53 mutation is common in HNSCC, strategies have evolved to target p53 mutant tumors. One approach has been to develop an adenovirus with the E1b 55 kDa gene deleted (ONYX-O15), engineered to selectively replicate and lyse p53-deficient cancer cells and spare normal cells (67). Selective intratumoral replication and tumor-selective tissue destruction have been documented in Phase I and II clinical trials of intratumoral injections of ONYX-O15 with or without chemotherapy in patients with recurrent or refractory HNSCC (68, 69). Although p53 mutant tumors were more likely to experience necrosis than p53 wild-type tumors when ONYX-O15 viruses were administered alone (69), the response rate appeared to be independent of the tumor p53 status when the viruses were injected together with systemic chemotherapy (68).

On the basis of these early encouraging results, a Phase III randomized study is being mounted to evaluate the efficacy of ONYX-O15 in addition to cytotoxic chemotherapy in recurrent HNSCC.

Another approach of targeting p53 mutations involves gene replacement using a replication-defective adenoviral vector containing the wild-type p53 gene (Ad-p53 or RPR-INGN-201). In preclinical studies, Ad-p53 vector system has been shown to induce apoptosis in neoplastic cells regardless of their p53 status and to reduce tumor growth in mouse xenografts (70). It has been tested in a Phase I clinical study, which involves preoperative and perioperative injections of the adenovirus in resectable recurrent HNSCC, and repeated intratumoral injection of the virus alone in patients with unresectable recurrent tumors (71).

Injections were well tolerated with injection site pain and fever being the most common side effects. Because some antitumor responses were observed in this dose escalation study, a large international Phase II study combining the virus with cytotoxic chemotherapy is in progress to determine its efficacy in the setting of recurrent/metastatic HNSCC.

**Targeting the Cell Cycle.** Cyclin D1 is commonly over-expressed in HNSCC, and p16, the endogenous inhibitor of CDK4, is commonly deleted as an early event in HNSCC progression (5). Flavopiridol, a CDK inhibitor, has been shown to repress the transcription of cyclin D, induce cell cycle arrest in G2 and G1 phases, and promote p53-independent apoptosis in preclinical experiments (72). It also enhances chemosensitivity of tumor cells 

Prevention of Secondary HNSCC

HNSCC patients who have been successfully treated remain at a high risk for developing additional neoplasms within the aero digestive tract. The lifetime risk for developing a SPT is estimated to be 20%, and the annual rate is 4–6% (79). SPTs have become the leading causes of death in early stage-treated HNSCC patients. Therefore, novel strategies to screen for or to prevent new epithelial cancers are highly desirable.

**Early Detection.** There is great promise for the role of molecular detection of HNSCC. Studies have shown that clonal genetic alterations can be detected in the blood and saliva of HNSCC patients. For example, the pattern of promotor hypermethylation, a mechanism for inactivating tumor suppressor genes, similar to those found in primary HNSCCs was found in DNA from saliva or serum of the same HNSCC patients (80, 81). A high frequency of somatic mitochondrial DNA mutations identical to those found in primary tumors was also noted in the saliva of 13 HNSCC patients (82). The clonal nature and high copy number of these mitochondrial mutations may provide an exciting method of screening for HNSCC from saliva. More recently, the use of serum proteomic patterns generated from SELDI-TOF mass spectroscopy provided a novel approach for
detecting ovarian cancer with a high sensitivity and specificity (83). This same technology may serve as a useful tool for the detection of HNSCC in high-risk patient population and for prognostication of those already with invasive tumors. Fig. 2 shows representative SELDI-TOF mass spectra of plasma samples from control and HNSCC patients (Fig. 2A) and “gel view” representation of SELDI-TOF spectra of plasma samples from patients with and without tumor recurrence (Fig. 2B).

**Chemoprevention.** A number of potentially effective chemopreventive agents have been studied or are awaiting clinical trials. Notable compounds include the retinoids, COX inhibitors, selenium, α IFN, and targeted therapies such as FTI or EGFR tyrosine kinase inhibitors (79). One group of compounds is the retinoids, which activate retinoic acid receptors, proteins that are important for the growth and differentiation of squamous cells. 13-cRA has been shown to reverse premalignant head and neck lesions in a Phase III randomized trial, although the remission was short lived (84). This compound was subsequently studied in a Phase III placebo-controlled, double-blind randomized study in previously treated HNSCC patients as an adjuvant chemopreventive agent to prevent SPTs. The use of 13-cRA significantly reduced the incidence of SPTs without affecting the rate of primary tumor recurrence (85). As none of the SPTs occurred during the active treatment period, long-term
treatment may be necessary. On the basis of these data, a large intergroup Phase III study involving >1000 patients comparing a 3-year regimen of low dose 13-cRA to placebo for SPT prevention in patients with treated early stage HNSCC has completed accrual and the data may be available as early as this year. The results of this trial, if positive, will have a significant impact in the management of HNSCC patients.

For patients who have completed treatment for locally advanced stage III-IV tumors, the combination of 13c-cRA, IFN-α, and α-tocopherol has yielded impressive results as adjuvant treatment after definitive therapy. In a Phase II study, patients who have received this biological combination for 1 year after active treatment had a survival rate of 91%, a loco-regional relapse rate of 9%, and a SPT development rate of 2% at 2 years (86). A Phase III study is ongoing.

COX-2, an enzyme that catalyzes the synthesis of prosta-glandins, is overexpressed in a variety of premalignant and malignant conditions, including those in the head and neck, and levels of COX-2 are increased in normal-appearing mucosa adjacent to HNSCC sites (87). Selective inhibitors of COX-2 can reduce the formation, growth, and metastases in a variety of experimental tumors, including HNSCC (88). These findings suggest a role of COX-2 in HNSCC carcinogenesis and progression, and the potential application of COX-2 inhibitors in prevention of SPT development. At the present time, selective COX-2 inhibitors are being evaluated as chemopreventative agents in patients with oral leukoplakia and Barrett’s esophagus. A major objective of these clinical trials is to determine whether such an agent can induce regression or reversal of these premalignant lesions.

Because mutation of the p53 gene appears to be an early event in the development of HNSCC, targeting p53 mutated cells or replacement of the p53 gene is another strategy for chemoprevention. ONYX-015 virus has been used in a mouth wash preparation to treat clinically apparent premalignant lesions with preliminary evidence showing regression of dysplasia (89). Additional work is needed to establish the role of p53 gene therapy in the prevention of SPTs.

Human Papillomavirus Vaccination. Human papilloma-virus serotypes 16, 18, 33, and 73 may have etiological relevance for oropharyngeal cancers, specifically tonsillar cancers (90). For these tumors, vaccination may be the only possibility for prevention. Prophylactic HPV vaccines against plain VLPs, which are DNA-free capsids containing the major viral capsid (L1) protein, or chimeric VLPs, which contain various combinations or early viral proteins attached to L1, are being tested in Phase III trials, specifically in young females for the prevention of cervical cancers. Data from Phase I-II trials showed that HPV VLPs were safe and highly immunogenic (91). A recently reported Phase III trial showed that vaccination with HPV-16 L1 VLP in young women can reduce the incidence both of HPV-16 infection and HPV-16-related cervical intraepithelial neoplasia (92). Overall, prevention of HPV infection may result in a reduction of the incidence of oropharyngeal cancer in young patients and possibly a decrement in HNSCC-related mortality.

Future Challenges

The era of genomics and proteomics together with the unraveling of the human genome have significantly improved the understanding of solid tumor physiology and resulted in rapid identification of new molecular targets for the diagnosis, prevention, and treatment of HNSCC. The new era raises new challenges for translating these new discoveries to the clinic. New head and neck animal models that closely resemble actual human tumors in their natural environment rather than the artificial xenograft systems that are currently being used are urgently needed for the understanding of the biological significance of newly discovered genetic pathways and for optimal evaluation of new therapies. Innovative clinical trial designs, which incorporate serial novel noninvasive surrogate endpoints such as molecular makers or imaging methods, for evaluation of responses and late toxicity are critical for application of new drugs. Better and more cost-effective approaches are needed to combine new targeted therapies with existing conventional treatments, and to define the optimal doses and treatment sequence for these combinations. Finally, emerging evidence indicates that inhibiting a single aberrant pathway may not be sufficient for tumor control in solid cancers because of their efficiency in compensating for such inhibition, and targeting a combination of pathways as well as the microenvironment are necessary to improve the cure rate in HNSCC. A major challenge is determining how and when to combine these therapies for optimal tumor cell kill while minimizing the toxicity to fragile patients.

References


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