Identifying the risk factors for late-stage head and neck cancer

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"Exciting advances are occurring in the understanding of the molecular pathogenesis of late-stage head and neck squamous cell carcinoma."

Head and neck cancer (HNC) prognosis, like most cancers, depends largely on the stage of the tumor, but other factors related to lifestyle, such as smoking and alcohol consumption, comorbidity, access to care, and tumor biology, can also affect outcome. Clinical stage, the most important prognostic factor for patients with HNC [1], assembled through accurate mapping of the tumor for precise local (T), regional nodal (N) and distant (M) extent of the tumor, and assigned a pretreatment TNM stage, is critical before commencing therapy [2]. Periodic revisions of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) TNM staging system used for staging HNC worldwide [2], include advances in diagnosis (e.g., endoscopy and radiologic imaging) as well as progress in improving our understanding of the biologic behavior of tumors arising in specific head and neck sites. The latest version has undergone significant modification unlike many other cancers and underscores HNC as a heterogeneous disease, both at the molecular and clinical level [2].

Accurate and reliable stratification of HNC for prediction of outcomes has been challenging, mainly because of the numerous anatomic sites and subsites from which tumors can arise and the diversity of histologic types of tumors in these locations [2]. The overwhelming majority of mucosal HNCs are squamous cell carcinomas (HNSCCs), affecting more than 500,000 people worldwide each year, accounting for 5% of all malignancies [3]. In the USA, approximately 52,140 new cases are expected in 2011 with an estimated 11,460 deaths for HNC of the oral cavity, pharynx and larynx [4].

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Despite considerable efforts, the 5-year survival rate for HNSCC has not changed significantly. Early stage (I and II) patients have a 60–95% chance of cure with local treatment alone, but patients with more advanced disease have a greater than 50% risk of recurrence or development of distant metastatic disease [3]. Lymph node metastases and distant metastases are the most important predictors of prognosis [3]. Many patients present with clinically advanced disease, where surgery, radiation and chemotherapy are the standard of care. Given the highly disfigurative nature of HNSCC surgical treatment and typically repeated exposure to

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high-dose radiation, identification of primary HNSCC tumors with enhanced metastatic potential by molecular means can aid clinicians in tailoring appropriate treatment strategies, especially in cases that have no apparent nodal involvement. Improvements in our ability to diagnose, evaluate and stage these patients can improve individualization of treatment.

Risk factors for HNSCC

In addition to tobacco and alcohol [5], epidemiological and laboratory evidence now warrant the conclusion that the human papilloma virus (HPV) is a causative agent for some HNSCC [6] and an independent risk factor for oropharyngeal cancer (OPSCC) [7]. The biologic significance of HPV as another independent risk factor is underscored by the improved prognosis for patients with HPV-positive HNSCC relative to HPV-negative HNSCC [7,8], due in part to a better therapeutic response to chemoradiotherapy [9].

> "...head and neck squamous cell carcinoma disparities as likely due to social rather than biological factors."

Head and neck SCC has a high mortality rate and disparate unfavorable diagnosis and prognosis outcomes for African–Americans (AAs) [4,10,11]. There is no consensus on the causes of the differences in the higher incidence of and the mortality from HNSCC for AAs when compared with Caucasian–Americans (CAs), but they can include differences in access to care, stage at diagnosis, insurance status and attitudes of health providers, as well as HPV infection status [12]. A recent study found that poorer survival outcomes for AAs versus CAs with OPSCC were attributable to racial differences in the prevalence of HPV-positive tumors. HPV positivity was higher in CAs (34%) as compared with 4% in AAs and HPV-negative AA and CA patients had similar survival outcomes [12].

Identifying the risk factors for late stage HNSCC

Race/ethnicity as a factor for late stage HNSCC

There is abundant epidemiological evidence that self-identified race/ethnicity is associated with differences in cancer incidence and mortality. The 5-year relative survival is lower in AAs than in CAs for every stage of diagnosis for nearly every cancer site [13]. For HNSCC, the disproportionate increase in the number in AAs as compared with CAs [14] also extended to disease stage [15], with a greater proportion of advanced-stage cancers (stages III and IV) occurring among lower-income groups, including AAs [10,11].

Differences in genetic background among study individuals can impact the power and reliability of genetic association studies; therefore, accounting for such variance is becoming more of an issue [16]. Methods to detect and control for differences in ancestry in genetic association studies utilize ancestry informative markers (AIMs), which have high utility in biomedical research because they can be used to accurately measure individual ancestry (IA) of enrolled study subjects [16]. Most importantly, these IA estimates can be used to control for heterogeneity in genetic studies in admixed populations such as AAs and Hispanic Americans [16,17]. Our recent study examined AIMs to estimate the amount of population admixture and control for this heterogeneity for stage and survival in a primary HNSCC cohort [17]. Results showed that only self-reported race as AA was associated with late stage. Stratification within the AA group by West African genetic ancestry revealed no correlation with stage or survival, pointing to the causes of HNSCC disparities as likely due to social rather than biological factors. These findings have clinical relevance for evaluation of health disparities. Given the heterogeneity in the AA population, genetic ancestry rather than self-reported racial designation can reduce potential confounding effects due to population admixture and control for heterogeneity, bringing into sharper focus, ethnic differences for cancer diagnosis and prognosis.

HNSCC

Molecular staging of HNSCC: are we there?

A current shortcoming in the prognosis and treatment of HNSCC is a lack of methods and large study cohorts to adequately address the etiologic complexity and diversity of the disease. Late stage HNSCC is limited to a complete response of 50% in contrast to an early stage diagnosis with 80% survival, highlighting the urgency of molecular markers with potential to unravel the pathogenesis of HNSCC.

Of the numerous studies published on various molecular markers as predictors for advanced-stage HNSCC, two reliable diagnostic and risk stratification strategies involve HPV and the EGFR status of tumor tissues. The association between HPV and HNSCC (for both incidence and prognosis) is strongest for OPSCC. HPV-positive OPSCC has been noted as a distinct variant of HNSCC characterized by high prevalence of HPV infection, better patient outcome, nonkeratinizing histology and overexpression of p16 [18]. Currently, HPV status is the most valid and robust molecular diagnostic and prognostic biomarker to date for HNSCC [19]. Despite the fact that HPV-positive HNSCC are more likely to be detected as late-stage cancers, survival has been shown to be better for patients with HPV-positive when compared with HPV-negative HNSCC, underscoring HPV as a reliable biomarker that can be used to not only help diagnose HNSCC, but to also risk stratify patients and help direct treatment plans based on the disease behavior and prognosis [9].

Patients with EGFR overexpression have a higher stage, increased lymph node metastasis, and shorter relapse-free survival and overall survival [20]. However, as a predictive biomarker for therapy responses, data are not definitive and problems associated with these molecular therapies and their mechanism of action need further study.

Other observed markers in HNSCC are loss of heterozygosity/microsatellite instability at 3p, 9p, 17p and 18q chromosomal locations [21]. Patients with benign premalignant lesions that harbored HNSCC-specific genetic losses and loss of heterozygosity had a significantly increased risk of developing cancer [22].

Mutations in the tumor suppressor *p53* gene occur in 45–70% of HNSCC and strategies targeting the *p53* gene and protein may halt or reverse the process of tumorigenesis [23]. Another important gene

product in HNSCC pathogenesis is the p16INK4a (p16) protein made by the p16 INK4a (*CDKN2A*) gene located at 9p21. p16 is a cyclin-dependent kinase inhibitor that inhibits phosphorylation of the retinoblastoma protein (pRb) and blocks cell cycle progression at the G1 to S check point [24]. Loss of p16 expression by deletion, mutation, or hypermethylation is common in HNSCC [25,26] and is associated with worse prognosis in some HNSCC [27]. On the other hand, p16 overexpression has been correlated with improved outcome in OPSCC [28]. This occurs as a result of functional inactivation of pRb by the HPV E7 protein resulting in the upregulation of p16 [9]. Thus, HPV-positive tumors are characterized by high expression of p16 with good evidence that p16 positivity may be a biomarker for tumors harboring clinically and oncogenetically relevant HPV infections [9,29].

> "Human papilloma virus testing is becoming part of a molecular staging system for head and neck squamous cell carcinoma, signaling that molecular staging in head and neck squamous cell carcinoma is here."

Gene transcriptional inactivation via hypermethylation at CpG islands within promoter regions is an important mechanism [30]. In primary HNSCC, promoter hypermethylation of *RARB* and *APC* in early-and late-stage tumors and of *CHFR* only in late-stage tumors suggested *CHFR* as a putative diagnostic biomarker for late-stage disease [31]. In a retrospective multi-ethnic primary laryngeal carcinoma cohort, aberrant methylation of *ESR1* was an independent predictor of late-stage laryngeal squamous cell carcinoma [15]. DNA methylation patterns also have utility in determining whether a second tumor represents a recurrence of the original malignancy or a second primary cancer [32].

Challenges to identification of risk factors for late stage HNSCC

The practical application of the discovery of reliable diagnostic and prognostic makers in HNSCC is starting to impact diagnosis and treatment of HNSCC to achieve a more personalized and effective approach. However, the testing of known as well as novel molecular markers as robust biomarker tools in molecular diagnosis needs to be undertaken in large, multi-ethnic, racially diverse and heterogeneous cohorts with respect to adequate representation of different HNSCC sites. In addition, to better understand the complex interplay of risk factors that can determine stage (diagnosis) at presentation, there is a need to develop statistical approaches to modeling the contributions and potential interactions of relevant risk factors. To be translatable into clinical practice, comprehensive statistical models to evaluate risk factor determinants of outcome require validation.

Comprehensive molecular modeling for late-stage risk factors

There is a dearth of large multi-ethnic studies of primary HNSCC patients. In addition, a majority of studies have examined HNSCC outcomes for diagnosis in narrower contexts of pathologic and clinical risk factors. The large multi-ethnic Detroit HNSCC study cohort of over 689 primary HNSCCs took a broader, more inclusive, approach by examining not one or two risk factors but instead looking at the many intertwined variables influencing health and disease to understand the contribution of tumor genetic alterations, pathologic and patient factor determinants in HNSCC diagnosis outcomes [33]. This racially diverse cohort permitted comprehensive modeling of demographic (three variables), histopathology (ten variables) and clinical/epidemiologic risk factors (nine variables) for 22 patient risk factor variables (non-gene variables). In addition, DNA from tumor and normal tissue from paraffin-embedded tissue blocks was interrogated for loss and gain of 113 unique genes using the multiplex ligation-dependent probe amplification assay (MLPA). The MLPA assay is ideally suited for DNA from formalin fixed paraffin-embedded tissue [25]. The contribution of tumor genetic alterations, pathologic, and patient factors was examined using logistic regression and for optimal model development, two-thirds of the cohort was used as the learning set for building diagnosis models and the remaining one-third for model validation. The model's predictive measure (receiving operator curve) was 76.3% based on the learning set and 76.5% based on the testing set (validation). The six independent (multivariate) risk factors for late stage (diagnosis) included loss of *CTPS*, gain of *IL10*, marital status married), versus not married, pattern of invasion, presence of perineural invasion, and tumor location in the hypopharynx and oropharynx. Race was not a factor for stage in this comprehensive integrative modeling process. These results most likely reflect several unique aspects of this study and include:

- The relatively large cohort of primary HNSCC with 42% AA;
- The primary healthcare environment of the Henry Ford Health System;
- Inclusion of a comprehensive array of risk factors, including tumor genetic alterations;
- Molecular modeling strategies to include model development and validation;
- In this Detroit cohort, health insurance type was noted for 87% of the patients; 88% of AAs and 87% of CAs.

These results are consistent with previous studies [34] that underscore the impact of insurance coverage on stage at diagnosis, highlighting the urgency of reducing/erasing health disparities in HNSCC through public-health efforts to improve access to high-quality cancer prevention, early detection, and treatment services.

Summary

Head and neck SCC comprise a wide spectrum of neoplasms with different tumor biologies, prognosis and response to therapies. Current tumor classification is based on morphology and anatomic distribution, which leads to a homogeneous treatment for different diseases. Moreover, traditional diagnostic methods such as clinical assessment, histopathological examination

and standard imaging techniques are limited in their capacity to provide information on prognosis and decision making. New imaging modalities, such as PET, may improve our ability to optimally stage tumors in the head and neck region. Insurance and cost-related barriers to care are also critical to alleviating cancer disparities and leveling the playing field to ensure that HNSCC patients have access to high-quality cancer prevention, early detection and treatment services. Exciting advances are occurring in the understanding of the molecular pathogenesis of late stage HNSCC.

Comprehensive modeling can expose high-risk predicators of late stage HNSCC, offering molecular markers for earlier detection in body fluids such as blood and saliva with the intent to improve screening accuracy and cost–effectiveness of diagnostic testing. HPV testing is becoming part of a molecular staging system for HNSCC, signaling that molecular staging in HNSCC is here. An expanding repertoire of robust diagnostic biomarkers that predict the likelihood of local tumor recurrence and/or development of distant metastatic disease is bound to be impetus for modification of the current AJCC–UICC staging system to reflect these important advances.

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