Validation of LOH Profiles for Assessing Oral Cancer Risk

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Abstract

This perspective examines the report by Zhang et al. in this issue of the journal (beginning on page *XiXiX*) on the validation and refinement of a set of risk markers for oral premalignant lesion progression that incorporates loss of heterozygosity (LOH) markers. The perspective also discusses some of the challenges and opportunities of incorporating predictive biomarkers into monitoring and refined enrollment criteria for prevention studies.

With an annual incidence of >500,000 cases, squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common malignancy in the world today (1). The major etiologic factors for this group of diseases include chronic exposure to tobacco and alcohol as well as infection with certain high-risk subtypes of the human papilloma virus (HPV; refs. (2–8). Despite advances in treatment, long-term survival from this disease, particularly when associated with tobacco and alcohol, has remained modest. The several factors associated with this poor outcome include delayed diagnosis. Although the long-term survival for early-stage disease is ~80%, late-stage SCC has a 5-year survival rate of only ~20%. Furthermore, "field cancerization" of the upper aerodigestive tract has the negative impact on overall survival of giving rise to second primary tumors, the most common reason for early-stage HNSCC treatment failure (9,10). Therefore, screening, early detection, and prevention are likely to be critical components for improving the management of this aggressive disease.

Cancer screening can be defined as the application of a test or tests to asymptomatic individuals in order to differentiate those more likely to have the disease from those who are unlikely to have it. It is believed that screening and early detection can decrease cancer-related morbidity and mortality. This belief is based upon the argument that late-stage cancers can be identified earlier in the disease process, when they can be treated more effectively. Particularly in situations where precursors to invasive cancer can be identified and removed (e.g., colorectal adenoma removal by colonoscopy or sigmoidoscopy), the collective medical opinion and practice has been to recommend screening. Only recently, however, have randomized trials shown that screening in the lung and colorectum can actually reduce cancer mortality by 20% and 26%, respectively (11,12). The hope is that effective screening and early detection will also reduce HNSCC morbidity and mortality. To date, however, the United States Preventive Services Task Force has not issued a

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recommendation for HNSCC screening because "the evidence is insufficient to recommend for or against routinely screening for oral cancer."

Currently, the conventional visual and tactile exam (CVTE), in conjunction with a punch/ scalpel biopsy of visually identified abnormalities, is the gold standard for HNSCC screening. The CVTE/biopsy has its limitations, however. First, the criteria for identifying and grading oral dysplasia are controversial, highly subjective, and open to a wide range of interpretation (13, 14). In addition, the histopathologic diagnosis of dysplasia is an imperfect predictor of malignant transformation. Lesions are considered precancerous when displaying atypical cytologic and morphologic features. The timing of malignant transformation, however, is both highly variable and poorly understood. Furthermore, only a portion of these lesions will ultimately undergo transformation, and there are no definitive histopathologic or molecular criteria for predicting the risk of malignant transformation of individual dysplastic lesions. Therefore, conventional histologic findings can only be utilized to indicate that a given lesion may have malignant potential.

Several recent studies underscore this dilemma. Holmstrup et al. reported that the presence of dysplasia did not influence the risk of developing SCC for patients with a diagnosis of oral dysplasia (15). Similarly, a retrospective study of 207 patients with dysplasia found that 39% of the lesions regressed, 20% remained stable, 33% developed new dysplastic lesions, and 7% developed oral SCC during a one-year follow-up (16). Last, a meta-analysis of 14 studies comprising 992 patients found a malignant transformation rate of 12% and a time to malignant transformation of 4.3 years (17). Importantly, a subgroup analysis of histologic grade of dysplasia showed no significant differences in time to malignant transformation. These findings emphasize that, at the present time, we cannot accurately predict malignant progression solely based upon conventional histopathology. Therefore, the inclusion of molecular biomarkers capable of stratifying oral premalignancy patients into low- and high-risk categories for progression to oral cancer would dramatically improve our ability to diagnose and treat oral premalignancy. At the present time, there are no validated such biomarkers that are considered standard of care. Several candidates, however, are worthy of further investigation (18,19). A consensus has emerged that loss of heterozygosity (LOH) at chromosomes 3p and 9p occurs at a high rate of frequency in both oral dysplasia and SCC (18–27). Until now, whether LOH at 3p/9p had sufficient diagnostic accuracy to sufficiently predict risk of progression to SCC had not been conclusively established.

As reported in this issue of the journal (Zhang et al, Loss of Heterozygosity (LOH) profiles-validated risk predictors for progression to oral cancer. Cancer Prev Res 2012;5:XiXiX) Zhang et al. sought to validate their original retrospective LOH model (25) and to further refine it by adding two additional LOH markers. Using a prospective cohort of 296 subjects with a histologic diagnosis of primary mild/moderate dysplasia, the authors first validated their "2000 model" for predicting progression to severe dysplasia, carcinoma in situ, or invasive cancer. Consistent with the original study, the high-risk (3p and/or 9p LOH) lesions were found to have a 22.6-fold increased risk of progression when compared with the low-risk (3p and 9p retention) lesions. Since only 20% of the cases with 3p and/or 9p LOH progressed in the original model (25), however, there was a need to identify additional markers capable of better stratifying these lesions. To further refine the LOH model, the authors used recursive partitioning analysis to construct a new classification model that added two additional markers (4q and 17p). This analysis allowed them to further stratify lesions into low-, intermediate-, and high-risk lesions. Using this algorithm, the intermediate- and high-risk groups demonstrated a 11.6- and 52.1-fold increased risk of progression, respectively, compared with the low-risk group. To validate the new model, they reclassified the cases from the retrospective cohort from the original "2000 model" with similar results to the prospective cohort. Finally, they combined both cohorts (prospective

and retrospective) to increase to sample size to over 400 subjects and repeated the analysis. For the combined groups, the 5-year progression rates for the low-, intermediate-and high-risk groups were 4.8%, 22.9% and 65.4%, respectively. In the low-risk group, no subject developed invasive cancer within the median follow-up period of 44.6 months.

This is an important study for several reasons. First of all, it clearly establishes that low-grade lesions demonstrating retention of 9p have approximately a 5% risk of progression to severe dysplasia or more-advanced disease over five years. This extremely low risk of progression would suggest that individuals who fall into this category do not require aggressive treatment or monitoring despite having a histologic diagnosis of dysplasia. This finding has clear implications for standard of care in following such lesions, although the recommended frequency of follow-up remains to be determined. Conversely, the high progression rate (approximately 65%) for the high-risk lesions would suggest that this group should be aggressively monitored for clinical progression and would be the most ideal candidates to enroll in studies of novel surveillance strategies as well as chemoprevention trials.

An obvious goal for successful chemoprevention is the development of treatments that can be taken easily by at-risk individuals for prolonged periods of time with minimal side effects. Since most interventions are associated with some side effects, the toxicity profile of the intervention must be balanced with the cancer risk of the target population. As is often the case with three-tiered risk classifications, the risk-benefit balance will be least clear for the intermediate-risk group identified in the Zhang et al. study. Although a 22.9% risk of progression is significant, the considerably lower risk in the intermediate-risk group compared with the high-risk group suggests that the two groups should be studied separately in clinical trials and may benefit from different preventive strategies. The identification of individuals with the low-risk LOH profile, who are unlikely to benefit from any intervention and who therefore should not be included in chemoprevention trials, significantly shifts the risk-benefit balance of any contemplated intervention and will positively affect chemoprevention trial design in the future.

Several important questions arise from the current work. First, it would be important to know if the low- and intermediate-risk lesions that did progress demonstrated changes in their LOH profiles that more closely resembled the high-risk lesions. The answer to this question could have important biologic and therapeutic implications. If the profiles of the lower-risk lesions do change with biologic progression, this would suggest that one might be able to monitor for clinical progression in lesions based upon the LOH profile. Alternatively, if the LOH profile remained unchanged as the lesions progressed histologically/clinically, this suggests that a subset of premalignant lesions can progress independently of the current proposed LOH model. Second, many individuals with oral premalignant lesions present with multiple lesions or develop new lesions over time. It will be of interest to determine the relationship between the LOH profile and the risk of multiple or new lesions as well as the profile's relationship with progression in these "secondary" lesions. Since approximately one-half of HNSCC occur at a site distinct from the visually identified oral premalignancy (23), the value of the LOH profile as a cancer risk marker will partially depend on its ability to predict progression in the entire at-risk field. Third, since a significant percentage of individuals with oral dysplasia have a history of HNSCC, the predictive potential of the LOH profile in this setting will also need to be investigated. Last, not all loci of LOH have the same prognostic impact, suggesting that specific losses may be integral to carcinogenic progression. Further work will be needed to translate the mechanistic implications of the specific LOH profiles into preventive and therapeutic strategies.

In summary, the work by Zhang et al. represents an important next step in the validation of LOH profiles that identify individuals with dysplastic oral lesions who are at a high risk for the development of invasive oral cancer. It has direct implications for standard care in the community setting, identifying low-risk individuals who are not in need of aggressive monitoring or treatment. It also has the potential to enrich prevention and screening trials with the highest-risk populations who are most likely to benefit from intervention. This risk stratification holds the key to the development of personalized approaches to oral cancer prevention.

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