Editorial

Can Molecular Assessment Improve Classification of Head and Neck Premalignancy?

Li Mao[1]

Molecular Biology Laboratory, Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

The carcinogenic process is multistep and requires the accumulation of multiple genetic alterations in epithelial cells in the upper airway. These alterations allow the cells to obtain growth advantages over other cells and malignant phenotypes. It is believed that carcinogens such as cigarette smoke and alcohol are the major etiological factors for development of HNSCC[2] (1, 2). The concept of field cancerization has been established since 1953 and is based on the fact that the epithelial surface of the upper airway can be exposed to the same common carcinogens and thus possesses an increased risk of HNSCC development (3). Extensive genetic analysis of HNSCC as well as head and neck premalignancies in recent years has resulted in the identification of many common genetic abnormalities in these lesions. The fact that oral premalignant lesions such as leukoplakia are common and easily accessible makes them an excellent model for studying oral carcinogenesis. These lesions can exhibit a variety of histological features from hyperkeratosis, hyperplasia, and mild dysplasia to severe dysplasia. Although lesions with dysplastic changes generally carry a higher risk for developing HNSCC, the morphological changes alone are insufficient to inform us whether a lesion is a result of clonal growth of a genetically damaged cell or a result of a reactive response to stimulating factors. Because an accurate classification of oral leukoplakia according to the risk of developing HNSCC is crucial to determine appropriate treatment strategies, novel methods or assays are required to improve the current practice of classification.

In this issue of *Clinical Cancer Research*, Rosin *et al.* (4) report an important study that provides strong evidence supporting the role of genetic tests in augmenting histopathological evaluation of oral premalignancies. The genetic test evaluated in this study was microsatellite analysis, which applies a sensitive PCR technique and uses highly polymorphic microsatellite markers. The analysis was used to determine LOH in chromosome regions containing critical tumor suppressor genes that are frequently inactivated in HNSCC, such as the *p16* tumor suppressor gene at the 9p21 region and the *p53* tumor suppressor gene at the 17p13 region (5, 6). In an earlier study, Mao *et al.* (7) reported that a significant number of oral leukoplakia lesions exhibited LOH at chromosome 3p14 and/or 9p21, and those lesions with LOH carried a higher risk for developing HNSCC, suggesting a potential application of microsatellite analysis in predicting cancer risk of oral leukoplakia. However, the study included only 37 patients and tested only two critical chromosome regions.

Rosin *et al.* (4) took advantage of their centralized Oral Biopsy Service and the database of the British Columbia Cancer Registry and identified 116 patients who had oral premalignant lesions and who had been followed-up for a long period. Twenty-nine of the 116 patients developed HNSCC at the same anatomical sites as the primary premalignant lesions. Importantly, only patients whose oral lesions had moderate histological changes (hyperplasia or mild or moderate dysplasia) were selected for the study because the risk of developing HNSCC is very difficult to determine by histopathological evaluation in this type of patient population. It was found that almost all lesions that later progressed to HNSCC exhibited LOH at 3p and/or 9p regions, whereas lesions that lacked any LOH at the two regions did not progress (4).

However, a significant number of lesions with LOH at the two chromosome regions did not develop HNSCC. These results are consistent with previous observations by Mao *et al.* (7) and indicate a limitation of using these genetic abnormalities that often appear early in the oral carcinogenic process as the only markers for risk assessment. What is interesting in the study of Rosin *et al.* (4) is the significantly improved prediction of HNSCC development when LOH status at additional chromosome regions that often occur relatively late in oral carcinogenesis was integrated (4). Nearly 60% of the premalignant lesions with LOH at 3p and/or 9p plus LOH at any other region tested developed HNSCC, and among the lesions that later progressed to HNSCC, more than 70% exhibited such a LOH profile (4). It appears that LOH profiles can effectively augment routine histopathological evaluation of oral premalignant lesions.

Although the results reported in the study of Rosin *et al.* (4) are promising, caution is necessary in interpreting the data and the potential clinical implication. Because this is a retrospective study, unavoidable selection bias and uncontrolled management of the disease in the patient population often make drawing a final conclusion difficult. Therefore, prospective studies with proper study designs are necessary to validate these findings. Furthermore, sample size is still moderate in light of the requirement of analyses with many different LOH profiles. The larger the variables considered in a profile for analysis, the more cohorts are required to make any meaningful conclusion. Furthermore, microsatellite analysis may not be suitable to evaluate some lesions if their carcinogenic process involves mechanisms other than LOH at the chromosome regions examined. Other

Received 10/21/99; accepted 11/2/99.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Molecular Biology Laboratory, Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030.

[2] The abbreviations used are: HNSCC, head and neck squamous cell carcinoma; LOH, loss of heterozygosity.

molecular alterations, such as DNA hypermethylation in the promoter regions of certain tumor suppressor genes and abnormal protein expressions, may be integrated with microsatellite analysis and histopathology to form a comprehensive but practical panel of assays to classify oral premalignant lesions.

A major challenge we are facing now is that once an effective detection system has been established to identify oral lesions that are at high risk of developing invasive oral cancers, we should be able to find a cure for patients with these lesions. Because genetic lesions (cells containing clonal genetic alterations) are usually larger than clinical or histopathological lesions, and the boundaries between normal epithelium and genetic lesions are difficult to define, a simple resection of high-risk oral premalignant lesions is unlikely to cure the disease. Furthermore, additional asymptomatic genetic lesions may be present in the defected field, making it even more difficult to control the disease. In fact, a number of the patients in the study of Rosin *et al.* (4) developed HNSCC despite extensive excision of the premalignant lesions or chemotherapy.

Although the number of patients who underwent chemotherapy in this group of cohorts was not specified, it will not be surprising to see that short-term treatment with cytotoxic agents does not effectively eliminate preneoplastic cells because these cells, unlike fully malignant tumor cells, are often insensitive to these agents. Clonal genetic analysis may provide clues to determine whether clones carrying genetic abnormalities persist after chemotherapy. In a recent study, Mao *et al.* (8) reported that clonal genetic alterations could still be detected in many patients, despite the remission of morphological lesions in the oral cavity or larynx, even after treatment with a chemopreventive regimen, suggesting that prolonged treatment with tolerable preventive agents is necessary to eliminate scars of genetically defective clones in the damaged field of the oral cavity.

References

1. Decker, J., and Goldstein, J. C. Risk factors in head and neck cancer. N. Engl. J. Med., *306:* 1151–1155, 1982.

2. Nam, J., McLaughlin, J. K., and Blot, W. J. Cigarette smoking, alcohol, and nasopharyngeal carcinoma: a case-control study among U. S. whites. J. Natl. Cancer Inst., *84:* 619–622, 1992.

3. Slaughter, D. P., Southwick, H. W., and Smejkal, W. Field cancerization in oral stratified squamous epithelium: clinical implications of multicentric origin. Cancer (Phila.), *6:* 963–968, 1953.

4. Rosin, M. P., Cheng, X., Poh, C., Lam, W. L., Huang, Y., Lovas, J., Berean, K., Epstein, J. B., Priddy, R., Le, N. D., and Zhang, L. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin. Cancer Res., *6:* 357–362, 2000.

5. Reed, A. L., Califano, J., Cairns, P., Westra, W. H., Jones, R. M., Koch, W., Ahrendt, S., Eby, Y., Sewell, D., Nawroz, H., Bartek, J., and Sidransky, D. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. Cancer Res., *56:* 3630–3633, 1996.

6. Brennan, J. A., Boyle, J. O., Koch, W. M., Goodman, S. N., Hruban, R. H., Eby, Y. J., Couch, M. J., Forastiere, A. A., and Sidransky, D. Association between cigarette smoking and mutation of the *p53* gene in squamous-cell carcinoma of the head and neck. N. Engl. J. Med., *332:* 712–717, 1995.

7. Mao, L., Lee, J. S., Fan, Y. H., Ro, J. Y., Batsakis, J. G., Lippman, S., Hittelman, W., and Hong, W. K. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nat. Med., *2:* 682–685, 1996.

8. Mao, L., El-Naggar, A. K., Papadimitrakopoulou, V., Shin, D. M., Shin, H. C., Fan, Y., Zhou, X., Clayman, G., Lee, J. J., Lee, J. S., Hittelman, W. N., Lippman, S. M., and Hong, W. K. Phenotype and genotype of advanced premalignant head and neck lesions after chemopreventive therapy. J. Natl. Cancer Inst., *90:* 1545–1551, 1998.