

Predicting Progression of Oral Dysplasia—Letter

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There are several studies trying to show that LOH can be used to predict prognosis as well as to predict risk of malignant transformation of potentially malignant lesions. Among these studies, we read with great respect the results recently published by Zhang and colleagues (1), which prompted us to write this commentary.

Microsatellite PCR amplification seems to be one of the most commonly used method to detect LOH, although the post-PCR detection technique is not a consensus. LOH analysis using microsatellite markers is based on the identification of loss of polymorphic markers flanking tumor suppressor genes in tumor DNA compared with matched normal sample.

In their article, Zhang and colleagues (1) validated LOH profiles as risk predictors to malignant transformation of oral premalignant lesions. They report interesting results based on gel band intensity comparison. In 1992, the first studies of LOH analyses in tumors were conducted using PCR products electrophoresis followed by different protocols of gel band intensity comparison (2, 3). However, now one knows that this method may be tricky if compared with capillary electrophoresis. Interpretation of gel electrophoresis results might also become difficult due to the appearance of stutter bands, mainly when formalin-fixed paraffin-embedded samples are used (4). Contrarily, capillary electrophoresis following PCR amplification of microsatellite markers is a powerful automated method that uncovers LOH even when tumor sample preparation contains normal tissue contamination (5). In addition, this method has a higher sensitivity, was shown to be reproducible and user-friendly and it leads to an easy result interpretation (4).

In our modest experience, we have seen some examples of conflicting results between gel and capillary electrophoresis, and similar differences have been previously reported by others (4). In Fig. 1, we show examples of gel electrophoresis and matched capillary electrophoresis with divergent results. Underestimation of LOH on gel electrophoresis is a problem observed specially due to contamination of tumor sample by normal DNA. Another relevant caveat is gel interpretation leading to a conclusion of LOH and when

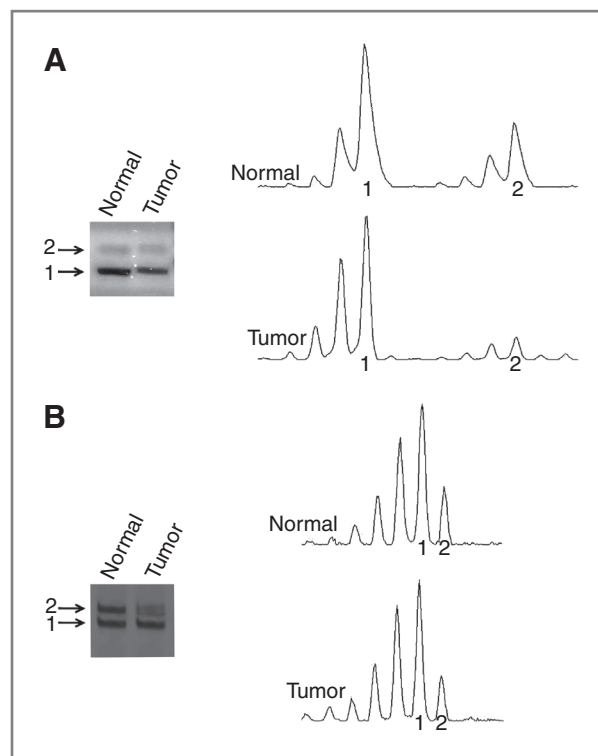


Figure 1. Examples of inconsistencies between gel and capillary electrophoresis results. On the left, we see the results of gel electrophoresis, whereas on the right we see screen shots of electropherograms generated by GeneMapper Software (Applied Biosystems) after capillary electrophoresis of the same PCR products that were run on the gel. Allele 1 is the short allele and 2 is the long allele. PCRs were carried out with the same DNA yield, both, normal and tumor under the same conditions. After capillary electrophoresis, LOH was calculated as the ratio between the short allele-normal (S_n)/long allele-normal (L_n) and short allele-tumor (S_t)/long allele-tumor (L_t) using the following formula: $(S_n:L_n)/(S_t:L_t)$. This step is automated. LOH was scored when one allele (peak) was decreased by more than 50% in the tumor sample compared with the same allele in normal tissue, followed by DNA stutter correction when necessary (score <0.5 or >2). A, polymorphic marker D9S169. Although the gel electrophoresis shows no evidence of allelic loss (i.e., retention of both alleles), the capillary electrophoresis analysis revealed LOH (score 0.44). B, polymorphic marker AFM238FW2. Although on the gel allele 2 of the tumor tissue is apparently decreased compared with its normal counterpart and could be interpreted as LOH, the capillary electrophoresis shows retention of both alleles (score 0.69).

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doi: 10.1158/1940-6207.CAPR-13-0072

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testing the same normal-tumor sample pair on capillary electrophoresis, one finds no evidence of LOH.

Our objective is to bring this interpretation issue to light. Misinterpretation may result not only in irreproducible results, but may generate spurious data. With increasing implications of LOH profiles to the patients with cancer, we must be cautious in interpretation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This study was supported in part by grants from FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) and by CNPq

(Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil.

Received February 26, 2013; revised March 1, 2013; accepted March 6, 2013; published OnlineFirst April 16, 2013.

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