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 et al (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 tumour suppressor genes in tumour DNA compared to matched normal sample.

In their paper, Zhang et al (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 tumours 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 to 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 tumour 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 figure 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 tumour sample by normal DNA. Another relevant caveat is gel interpretation leading to a conclusion of LOH and when testing the same normal-tumour 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 cancer patients, we must be cautious in interpretation.

Acknowledgements

CCG and RSG are research fellows of CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil
References


Figure Legend

Figure 1. Examples of inconsistencies between gel and capillary electrophoresis results. On the left we see the results of gel electrophoresis while on the right we see screen shots of electropherograms generated by GeneMapper Software (Applied Biosystems, CA, USA) 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 tumour under the same conditions. After capillary electrophoresis, LOH was calculated as the ratio between the short allele-normal (Sn)/long allele-normal (Ln) and short allele-tumor (St)/long allele-tumor (Lt) using the following formula: (Sn:Ln)/(St:Lt). This step is automated. LOH was scored when one allele (peak) was decreased by more than 50% in the tumor sample compared to the same allele in normal tissue, followed by DNA stutter correction when necessary (score <0.5 or >2) A) Polymorphic marker D9S169. While 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. While on the gel allele 2 of the tumour tissue is apparently decreased compared to its normal counterpart and could be interpreted as LOH, the capillary electrophoresis shows retention of both alleles (score 0.69)
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Cancer Prev Res Published OnlineFirst April 16, 2013.

Updated version

Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-13-0072

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