"Next Generation Sequencing (NGS) Copy Number analysis re-defines the classification of Oral Verrucous Carcinoma"

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Background:

Oral verrucous carcinoma (OVC) is categorised as a low-grade variant of oral squamous cell carcinoma (OSCC). The aetiology of OVC is unknown, and the suggested role of human papillomavirus (HPV) as a causative factor remains contentious. Distinguishing OVC from OSCC is a common problem for pathologists due to the poorly defined diagnostic criteria. The rarity of these lesions also makes them difficult to investigate, so most previous studies have been made on small numbers of cases. The aim of this study is to use NGS copy number (CN) analysis to identify the genomic characteristic features of oral verrucous lesions, including OVC, and oral verrucous hyperplasia (OVH), a possible precursor of OVC, and compare them with classical OSCCs, and to analyse those lesions for the presence of HPV.

Methods:

We identified a total of 57 OVCs, 16 OVHs, and 45 OSCC cases. DNA was extracted from all FFPE samples and sequenced at a coverage between 2.5% and 13%. Genomic CN karyograms were produced and compared between OVH, OVC and OSCC samples. All samples were analysed for the presence of HPV subtypes and for all known human viruses.

Results:

Visual inspection of patient's CN karyograms revealed that genomic signatures usually associated with OSCCs were completely absent in oral verrucous lesions. Gains of chromosome arms 7q, 16q and 17q were detected in OVCs at a frequency of ~50%, suggesting that these CN alterations may be involved in the development of OVC. Interestingly, losses were detected more frequently in OSCCs than OVCs, suggesting that these CN alterations may be related to the more aggressiveness behavior of OSCC tumours. Significant CN alterations in the OVH group were present in OVCs. An HPV-16 sequence was detected in one OVH and one OVC, and an HPV-2 sequence was detected in one OVC out of the 73 verrucous cases with viral loads of 2.24, 8.16 and 0.33 viral genomes per cell respectively.

Conclusion:

A previous study conducted by our group have shown that NGS can be used as a powerful method for detection of HPV subtypes and loads, and provide CN karyograms for FFPE samples in a single test. To the best of our knowledge, this is the first study to use NGS CN analysis and viral detection method on oral verrucous lesions. Although the WHO describes OVC as a variant of classical OSCC, our CN results suggest that it is a distinct entity. Nonetheless, and despite the verrucous appearance, which is suggestive of viral aetiology, our results indicate that there is no HPV involvement in oral verrucous lesions. We demonstrate that CN analysis could contribute to differential diagnosis of oral verrucous lesions and classical OSCCs using routine biopsy specimens.