Heterozygosity of p16 expression in an oral squamous cell carcinoma with associated loss of heterozygosity and copy number alterations

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Abstract

Background

Oral field cancerization describes a multifocal development process involving many cells at once in response to prolong exposure to carcinogens. This case demonstrated differential p16 expression in different sections of the same HPV negative tumor on the floor of mouth in a patient with history of prolonged smoking.

Methods

Histological examination, presence of HPV infection and OncoScan analysis of DNA extracted from two well-defined areas with different p16 expression profiles were performed.

Results

Histological and immunochemical analysis revealed the presence of a dual architectural pattern squamous cell carcinoma with a p16 negative and a p16 positive component. OncoScan analysis showed genetic changes that define field cancerization of the p16 negative tumor as revealed by mosaicism in both loss of heterozygosity and copy number alterations in cancer-associated genes located on 3p, 7p, 9p 11q, and 17p.

Conclusion

These changes were indicative of field cancerization in response to tobacco exposure.

KEYWORDS

carcinogenesis, field cancerization, OncoScan analysis, Oral cancer, p16 expression

1 INTRODUCTION

Overexpression of p16 protein is used as a surrogate marker for human papillomavirus (HPV) involvement in non-keratinizing squamous cell carcinomas of the oropharynx.[1] Patients with HPV positive oropharyngeal carcinomas have a significantly better overall and disease-free survival compared to patients with HPV-negative tumors.[2] Although the total mutational burden between HPV-positive and HPV-negative head and neck cancers is fairly similar, the spectrum of mutations differs significantly.[3]

Recent studies have shown that high-risk HPV's are implicated in a small subset of oral squamous cell carcinomas although there does not appear to be a prognostic benefit as is the case for HPV-associated oropharyngeal carcinomas.[4] Overexpression of p16 cannot be used to identify HPV-positive oral carcinomas. It has been determined that about 30% of oral squamous cell carcinomas express p16 but HPV could only be demonstrated in 4% of those through HPV in situ hybridization.[5]

This case report described a primary squamous cell carcinoma on the floor of mouth consisting of two well-defined components, one p16 positive and the other p16 negative. The genetic changes in these two areas were compared.

2 CASE REPORT

A 57-year-old man presented with an ulcerative lesion, 2 × 1.5 cm in diameter in the left floor of mouth area. The patient had a 40 pack year smoking history and was HIV negative. A clinical diagnosis of oral squamous cell carcinoma was made and an incision biopsy performed. The tissue fragment measured 10 × 5 × 4 mm on gross examination.

Histological examination showed the presence of a squamous cell carcinoma with a dual architectural pattern delineated by intervening stroma. The one component consisted of a moderately differentiated, non-keratinizing squamous cell carcinoma with small, focal areas of keratinization (Figure 1). Immunohistochemical (IHC) analysis for p16 expression (p16 purified mouse anti-human p16 [INK4] clone G175-405 antibody; BD Biosciences.com) was negative for this fragment (Figure 2). The second component showed more pleomorphism with tumor invasion through a more trabecular growth pattern. More keratin islands were present. The tumor was graded as a moderately differentiated keratinizing squamous cell carcinoma (Figure 1). Strong nuclear and cytoplasmic expression of p16 was present in all the tumor cells of this component (Figure 2). In situ hybridization using a cocktail of high-risk HPV DNA probe was negative (Ventana INFORM HPV III Probe (B); Ventana Medical Systems Inc., Tucson, Arizona).

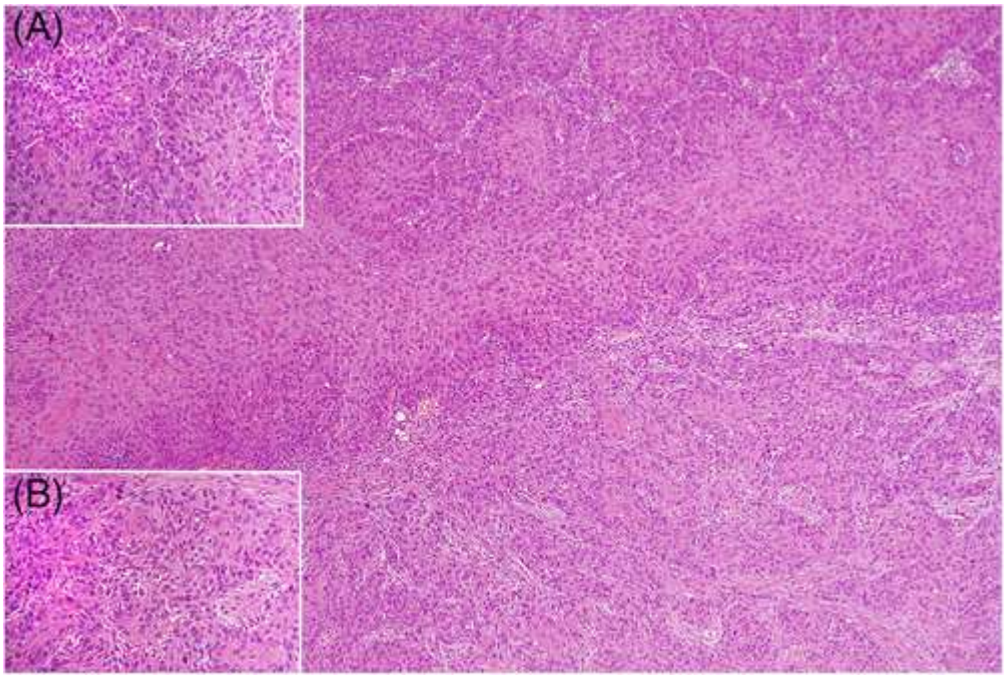

Figure 1. Low power photomicrograph showing a squamous cell carcinoma where the upper part consists of a moderately differentiated, non-keratinizing squamous cell carcinoma with small, focal areas of keratinization (hematoxylin-eosin stain; original magnification X40). A higher magnification is shown in insert A (original magnification X200). The lower part shows a more trabecular pattern with areas of keratinization. A higher magnification of this component is shown in insert B (original magnification X200)

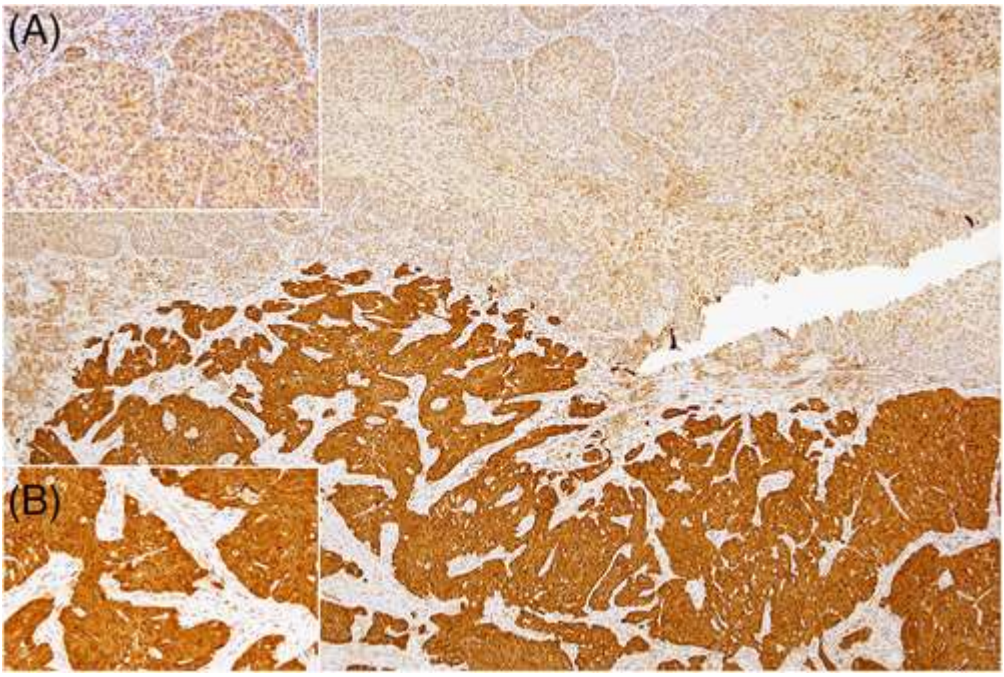

Figure 2. The heterogeneity of p16 expression is clearly visible (original magnification X40). Inserts A and B are higher magnifications of the two components (original magnification X200)

The p16 positive and p16 negative tumor areas in the formalin fixed paraffin embedded block, guided by the p16 IHC stained slide were separated with a scalpel blade and embedded in two separate paraffin blocks. p16 positivity and negativity was confirmed by repeating the p16 IHC on sections of both blocks. Twenty 10-µm sections were cut from each block and placed in separate Eppendorf tubes. DNA extraction was performed using

the QIAamp DNA FFPE Tissue kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The DNA was then submitted to OncoScan analysis.

The p16 positive and negative tumor areas showed two distinct patterns of chromosomal aberrations with OncoScan. The p16 negative area displayed mosaicism for all aberrations which was not the case with the p16 positive area. Mosaicism in the p16 negative area denotes the presence of genetically distinct populations of cells in this area with mutations of different scales that are propagated in only a subset of the cells of this individual. Whole genome view from OncoScan revealed more detectable chromosomal aberrations (loss of heterozygosity and copy number alterations) in the p16 negative compared to the p16 positive area (Figure 3). Furthermore, molecular profiling of p16 positive and negative areas showed differences in genetic changes in some key genes associated with oral cancer development (Table 1).

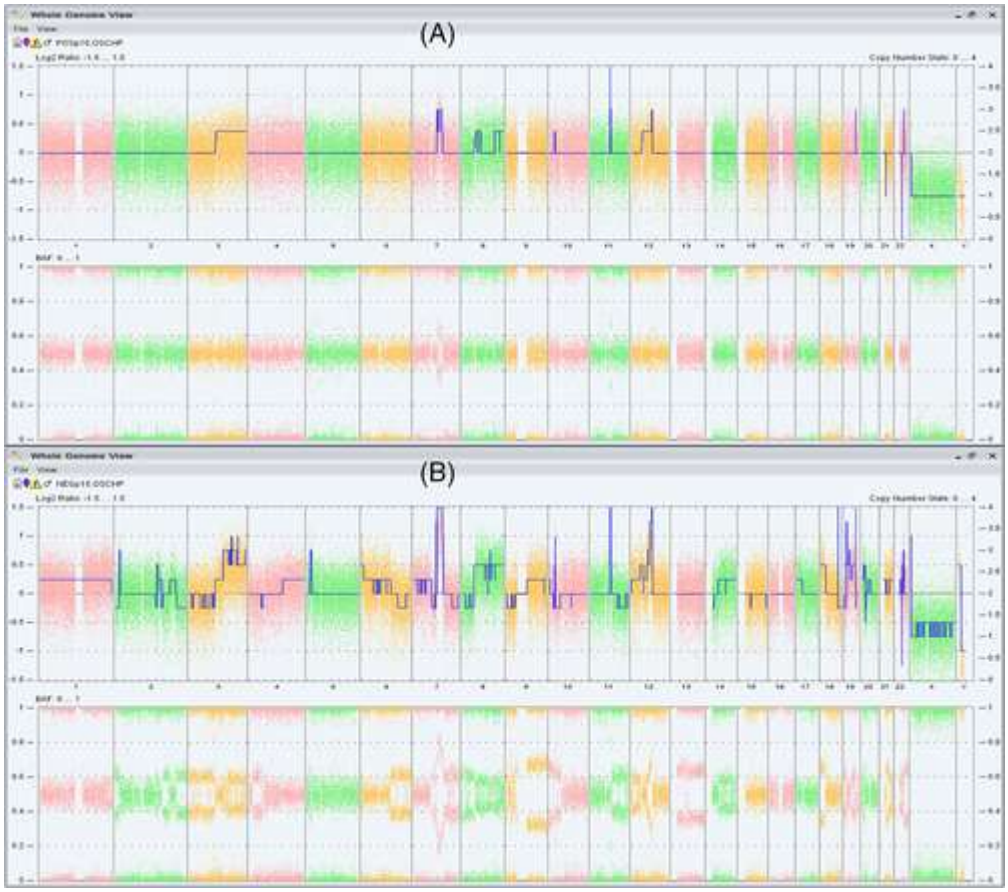

Figure 3. Whole genome view of p16 positive and negative areas of the tumor. The p16 positive area (A) showed a much clearer genomic view than the p16 negative area (B) which had many detectable chromosomal aberrations. Log2 ratio is use for interpretation of copy number state (CN) while BAF (B-allele frequency) together with log2 ratio are used to interpret loss of heterozygosity (LOH). A log2 ratio of zero denotes CN = 2 (diploid), while values above and below zero represented by the blue lines denote gain (CN > 2) and loss (CN < 2), respectively in that region. BAF is represented by three distinct tracks at 1, 0.5, and 0 representing the BB, AB, and AA allele. Lack of AB calls (absence of track at 0.5) represents a LOH in that region and can be distinguished from a loss by looking at the log2 ratio

Table 1. Difference in mutational landscape between p16 positive and negative areas of some known oral cancer associated genes

Chromosomal arm with aberrations	p16 positive, HPV-	p16 negative, HPV-
1p		NRAS (gain)
2q		CASP8 (gain), NFE2L2 (Cn-LOH), CUL3 (loss)
3p		Loss (FHIT and RAF1)
3q	Gain (TP63, SOX2 and PIK3CA)	Gain (TP63, SOX2, and PIK3CA)
4q		FAT1 (gain)
7p		EGFR (gain)
7q		BRAF (loss)
9p		Loss (CDKN2A, CDKN2B, and NFIB)
9q		Gain (NOTCH1 and PTCH1)
11q	Gain (CCND1, FADD and CTTN)	Gain (CCND1, FADD, CTTN, BIRC2, and ATM) and loss (YAP1)
12p		KRAS (gain)
13q		RB1 (Cn-LOH)
14q		AJUBA (loss), TRAF3(gain)
17p		TP53 (gain)
20p		E2F1 (gain)
22q	GSTT1 (loss)	GSTT1 (loss)

The p16 negative area showed mosaicism in all aberrations. Cn-LOH represents copy number neutral-LOH.

3 DISCUSSION

The Affymetrix OncoScan FFPE assay provides a comprehensive coverage of whole genome copy number and LOH analysis of genes that are well-known to be of significance and importance in cancer and tumor progression. It works efficiently on formalin-fixed paraffin-embedded (FFPE) tumor samples with DNA concentrations from as little as 80 ng. It was therefore used to look at the genetic differences between the p16 positive and p16 negative areas of this tumor. OncoScan revealed mosaicism in all aberrations detected in the p16 negative area but not in the p16 positive area. Mosaicism in p16 negative area denotes the presence of distinct subpopulations of cells with different mutations that are propagated in only a subset of the cells.

Both p16 positive and negative areas showed homozygous deletion of GSTT1 which has been linked to cancer susceptibility due to the inability of the glutathione S-transferase multigene family to eliminate metabolic carcinogenic compounds.[6] This could possibly explain the development of this cancer as a result of different mutation rates in the GSTT1 null squamous cells within the oral mucosal in response to tobacco exposure.

The amplification of the squamous lineage transcription factors (TP63 and SOX2) and candidate oncogenes (PIK3CA, CCND1, FADD, and CTTN) which are frequently altered in HNSCC[7, 8] was observed in both areas.

The expression of p16 is considered to be a surrogate marker for HPV infection, but, this is only true for oropharyngeal cancer[9] and is yet to be demonstrated in oral cancer. Therefore, it is not surprising that the p16 positive tumor area was negative for high risk HPV infection. This suggests the existence of a non-HPV-p16 expression pathway in this case of oral cancer. Furthermore, the p16 positive area clearly showed a molecular profile which is typical for oral cancer (Table 1). The p16 positive area is a typical TP53 wild-type-HPV negative tumor and this kind of tumor has a favorable clinical outcome compared to TP53 mutants.[8]

The p16 negative area displayed a mosaic gain of TP53 and other candidate oncogenes (EGFR, NRAS, and KRAS), which makes it a completely different tumor from that of p16 positive area that is TP53 wild-type. Mutation in TP53 has been used to define clonal relationship in oral field cancerization and the formation of patches (field precursor lesions) in tumor-adjacent mucosal epithelium.[7, 10] LOH in 3p and 9p observed only in p16 negative areas are early molecular changes in field cancerization which is reflective of early carcinogenesis and occur in dysplasia of squamous epithelium.[7] Field cancerization as first defined by Slaughter et al describes the presence of more than one independent area of malignancy in the tumor-adjacent epithelium in oral squamous cell carcinoma and exposure to carcinogen was suggested to be the main cause.[11] Slaughter et al then linked the occurrence of local recurrences and multiple primary tumors to dysplastic changes (fields) surrounding tumors in many oral cancer cases.[11] The TP53 mutation p16 negative area which is a tumor-adjacent mucosal epithelium confirms the findings reported by van Houten et al[12] of p53-positive focal patches in tumor adjacent epithelium which seems to precede the development of fields.

Mechanistically it can be proposed that the TP53 mutant patch cells or clonal unit either gain growth advantage and/or escape normal growth control and further acquire several different genetic aberrations such as in 3p (loss of FHIT), 7p (gain of EGFR), 9p (loss of CDKN2A), and 11q (gain of CCND1) to develop into an expanded field which eventually replaces the normal mucosal epithelium. In due course, a subclone in this field may acquire several other cancer-associated genetic changes to become an invasive tumor that will gradually progress to metastasis. This proposed model of field cancerization in the p16 negative area based on the molecular data is consistent with the integrated model for molecular development of field proposed by Leemans et al in a case of an HPV negative tumor with high chromosome instability,[7] which is faithfully recapitulated in this case.

4 CONCLUSION

The mutant TP53 tumor-adjacent p16 negative area with high chromosomal instability is completely different from the TP53 wild-type p16 positive tumor as revealed by molecular data, and is suggestive of carcinogen-induced field cancerization in this individual with a history of prolonged smoking.

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