p53: Revealing the Unusual Suspect: a Study and Field Cancerization Minireview

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Introduction

Oral carcinogenesis is a multistep process in which clinically recognizable prestages (leukoplakia and erythroplakia) occasionally occur [1]. Head and neck squamous cell cancer (HNSCC) is often associated with widespread epithelial histopathological alterations and despite successful treatment of a primary head and neck cancer, there is a high likelihood of occurrence of a second primary tumor similarly as seen in cases of upper aerodigestive tract and lung cancers [2]. These observations led Slaughter et al. [3] to propose the concept of “field cancerization”; the hypothesis that there are carcinogen-induced changes throughout the mucosa of the upper aerodigestive tract of head and neck cancer patients.

Originally, it was thought that all second primary tumors develop independently after widespread epithelial exposure to carcinogens [3]. In contrast, several other reports suggested that at least a proportion of second

primary tumors in HNSCC patients have arisen from one clonal cell population which is excepted presently [4, 5]. Various mechanisms have been proposed to explain this proposed common clonal origin of second primary tumors such as shedding of premalignant cells into the saliva and implantation at other sites [4, 6] or lateral migration of isolated premalignant cells [4, 5].

Braakhuis et al. [7] proposed the patch field carcinoma model to explain progression of genetically altered stem cell to carcinoma in normal oral mucosa. This genetically altered clone of cell expands laterally even greater than 7 cm in diameter [7]. With further more genetic hits in the predisposed area malignant transformation might happen at different areas of hits, which explain the concept of second primary tumor. Accumulation of mutations in oncogenes and tumor suppressor genes is a major genetic molecular mechanism of tumor development. Among the tumor suppressor genes, inactivation of the *p53* gene is one of the most frequent events [8].

Thus the aim of the present study was to evaluate the concept of field cancerization using p53 as a marker of field change in a case of patient who presented with multiple lesions ranging from premalignancy to malignant oral squamous cell carcinoma.

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Clinical Details

A 53 year old male patient presented with the chief complaint of pain and non-healing ulcer in the left back region of the mouth since last six months. Patient gave history of alcohol consumption and tobacco chewing since last 25 years.

On examination two lesions were observed. Ulcerative growth approximately 4×3 cm in dimension was present on the lower left retromolar area extending from the third molar to pretonsillar region (Fig. 1a). The growth was fixed to the

Fig. 1 **a** Photograph showing ulcero-proliferative growth in the left mandibular posterior alveolar region. **b** Photograph showing erythematous plaque on the hard palate separated from the ulcero-proliferative growth by normal appearing mucosa

underlying tissue. Another lesion appeared as a red plaque measuring about approximately 3×3 cm present in the mid line of hard and soft palate (Fig. 1b). Both the lesions are well separated by normal oral mucosa. Left submandibular lymph node was palpable, firm, non-tender and freely mobile over the underlying tissue.

The interesting aspect of the clinical presentation was the multiplicity of features, ranging from an ulcerated lesion in the retromolar region to an erythematous plaque extending over the palate, including the soft palate. A normal appearing mucosa was evident between the two lesional areas.

Histopathology and Immunohistochemistry (IHC)

Incisional biopsies were performed from the ulcerated lesion on left posterior body of mandible, erythematous palatal mucosa and the normal appearing mucosa present posterolaterally to the palatal lesion.

On histopathological examination of H&E stained slide the ulcerated lesion was diagnosed as Well differentiated squamous cell carcinoma (WDSCC) (Fig. 2a). Biopsy from erythematous area was reported as moderate to severe dysplasia (Fig. 2b). However the clinically normal mucosa exhibited no histopathological changes (Fig. 2c).

IHC was performed for all the three tissue sections using anti p53 antibody (DO7-Biogenex) which stains both wild and mutated p53 protein, utilizing streptavidin-biotin complex protocol. p53 was found to be strongly expressed in almost all the cells in OSCC showing dark intensity of staining (Fig. 3a). Intense p53 expression was noted in basal and spinous cells of moderate to severe dysplasia tissue as well (Fig. 3b). Uniform p53 expression was seen in the basal, suprabasal and initial spinous layer of cells in the histologically normal tissue (Fig. 3c). However intensity of staining was similar to OSCC and dysplastic lesion.

Discussion

The concept of field cancerization was first introduced by Slaughter et al. [3] in 1953, while studying the presence of

histologically abnormal tissue surrounding oral squamous cell carcinoma. It was an attempt towards the explanation of development of multiple primary tumors and local recurrence.

‘Field Cancerization’ or ‘Field Effect’ is used with context of existence of pre-neoplastic process at multiple sites, often with the unproven assumption that these have developed independently [9]. A variety of genetic changes are frequently detected in head & neck cancer for example squamous cell carcinoma which include chromosomal alterations; specific alteration of proto-oncogene and the tumor suppressor genes [10]. Among these p53 is the most effective tumor suppressor gene and usually gets mutated more often in oral cancers.

Multifocal tumors arise in the mucosa damaged by prolonged heavy exposure to alcohol and tobacco [11] as is true in the present case with history of alcohol and tobacco consumption for last 25 years. Recent molecular findings support a carcinogenesis model in which the development of field with genetically altered cells plays a central role.

Braakhuis et al. [9] proposed a genetic model of HNSCC. First a patch develops, consisting of clonal unit of p53 mutated cells (a stem cell and daughter cell) has been described in breast, lung, skin and HNSCC. The next step is conversion of the patch into a field, an epithelial lesion consisting of cells with cancer related genetic alterations, which expands on expense of normal tissue. During field progression number of genetic alterations takes place including loss of heterozygosity at chromosomal number 3p, 9p, 8p and 18q [12]. For the progression of field to cancer, the amplification of 11q13 has been shown to be important [13].

Nees M. et al. (1993) successfully identified p53 mutations in a small number of biopsies of normal mucosa of subjects with multiple tumors by sequencing abnormal migrating single strand conformation polymorphism (SSCP) PCR [14].

In the present study we have used mutated p53 as a marker for field change. p53 expression was noted in histologically diagnosed OSCC and moderate to severe dysplasia. P53 expression was seen in almost all the dysplastic cells in OSCC epithelium as well as epithelial islands. Moderate to severe dysplasia showed presence of p53 expression in basal and spinous cell layers which was in accordance to various studies and corresponds with clinically evident lesions.

Fig. 2 **a** Photomicrograph (H&E stain, 200×) depicting histopathological features of well differentiated squamous cell carcinoma. **b** Photomicrograph (H&E stain, 200×) exhibiting histopathological features of moderate to severe dysplasia. **c** Photomicrograph (H&E stain 200×) showing histologically normal oral mucosa

Clinically normal oral mucosal biopsy showed p53 positivity in the basal and suprabasal layers. The expression was uniformly present throughout the basal and parabasal layers. Normal (wild) p53 gene function is to prevent cell from accumulation of DNA damage. Wild type p53 protein activates the transcription of genes involved in G1 arrest, particularly the p21/waf1/CIP1

Fig. 3 **a** Photomicrograph (p53 IHC stain, 400×) revealing intense p53 positivity in all the tumor cells. **b** Photomicrograph (p53 IHC stain, 400×) showing intense p53 positivity in all cell layers of dysplastic epithelium. **c** Photomicrograph (p53 IHC stain, 400×) showing intense p53 positivity in basal and suprabasal cell layers of normal epithelium

gene; allowing repair. Wild p53 protein has very short half life to be detected histologically. p53 mutation is a fundamental mechanism involved in disordered growth control, and not a secondary consequence of neoplastic phenotype. In addition to abolishing the tumor suppressor activity of the protein, there is also stabilization of the protein, resulting in its accumulation to levels detectable by Immunohistochemistry [15].

Anti p53 antibody used in present study was DO-7 clone, which stains both wild and mutated p53 protein. Uniform p53 expression in basal cells indicates presence of predominantly mutated p53 as well as wild type p53. P53 expression above the basal cell layer in oral mucosa as in our case is an early event of malignant transformation and has a predictive value for developing OSCC.¹ P53 positivity in suprabasal layer indicates their existence in the uncontrolled mitotic pool due to p53 mutation.

The p53 positive cells showed intense (dense) staining all the three tissues which has more aggressive predictive value [16]. According to Slootweg [17], intense p53 expression in the cells correlates with higher proliferative activity of the cells. Thus the cells showing p53 positivity in oral epithelium is like an expanding patch of cells which on further genetic hits might convert into a field change.

Therefore it can be concluded that the present case is a typical example of “Field Cancerization Concept”. In that the variety of clinical and histological presentations are evident supported by p53 immunohistochemistry. Epithelium from the non-lesional area exhibited no dysplastic features was the unusual suspect; revealed by p53 which was used as marker for early field change. With genetic change and addition mutations the field mucosa could probably progress to carcinomatous lesion.

Thus the presence of field with genetically altered cells might be a risk factor for oral cancer. And that the p53 positivity in non lesional areas in carcinoma patient might be predictive for later occurrence of second primary tumor. Early detection and monitoring of field at risk and development of targeted molecular intervention may have profound implications for oral cancer prevention [7].

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