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Review - Study Group GIPaTeC

Oral field cancerization: history and future perspectives

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Summary

Notwithstanding extended surgical approaches or adjuvant chemoradiotherapy, the development of multiple neoplastic lesions arising in the oral cavity after treatment still represents a critical clinical challenge in the management of patients with oral squamous cell carcinoma.
Such clinical behavior of oral squamous cell carcinoma is nowadays better known as "field" cancerization effect as suggested by Slaughter, the author that for the first time tried to describe it in a scientific paper.
Field cancerization is now widely accepted not only in head and neck oncology but also in other anatomical districts as well as in different types of epithelial neoplasia. A brief history of the theory of field cancerization is here proposed and future perspectives deriving from new molecular techniques are discussed.

Introduction

The term "field cancerization" was introduced by Slaughter and colleagues in 1953 [1] to explain the appearance of multiple oral squamous cell carcinomas (OSCC) in the same patient to explain the appearance of multiple oral squamous cell carcinomas (OSCC)in the same patients. This model for head and neck cancer is still actual nowadays. Aim of the present work is to review the principles of Slaughter's theory of field cancerization and to illustrate what has changed in our knowledge and understanding in the last 60 years.

Slaughter's principles and first evidences

Analysing a population of 783 patients with OSCC Slaughter et al. [1] recorded in a publication titled "Field cancerization in oral stratified squamous epithelium. Clinical implications of multicentric origin" that 1) oral cancers usually had the tendency of spreading more easily in laterality than in depth 2) that the mucosa surrounding the neoplasia frequently harboured clinical or morphological atypia. 3) that OSCC may consist of multiple independent foci that eventually may converge 4) OSCC may develop multifocally in distant areas presenting preneoplatic features 5) the persistence of altered epithelium after surgical resection may induce the formation of new carcinomas.
A similar apparently independent multifocality, they acknowledged, was "well above the statistical possibility of chance occurrence, therefore they concluded that multiple OSCC should be the effect of a "field cancerization", in which an "area of epithelium has been preconditioned by an as-yet-unknown carcinogenic agent. Such a carcinogenic influence if operative long enough in time and intense enough in exposure produces an irreversible change in cells and cell groups in the given area, so that change of the process toward cancer becomes inevitable.
Nowadays we know that not only was Slaughter's intuition correct but also that it deeply helped our understanding of the natural history of OSCC. At that time, however, molecular techniques could hardly be performed hence it should not be surprising that a work citing the theory of field cancerization appeared no sooner than 16 years after Slaughter's publication.
In 1969 Roth et al. [2] studying the healing performances of UV-induced damaged cells acknowledged that epithelium from patients affected by oral and upper aero digestive tract squamous cells carcinoma showed a reduced repairing ability. They reported that a similar deficit could be the result of a damage at DNA level predisposing for further tumour development.

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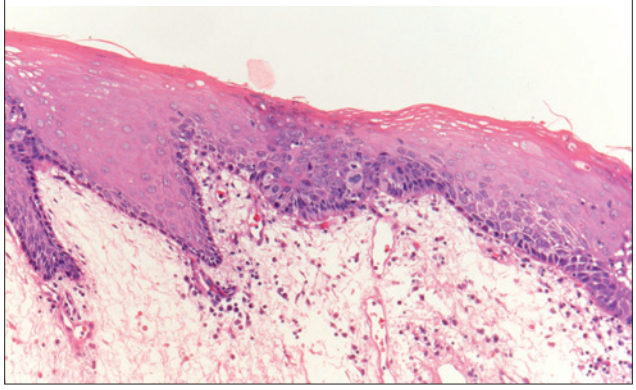
Albeit Slaughter's theory had gained some clinical evidence it still lacked proper scientific basis and it could not gain widespread popularity.
In 1982, Incze et al. [3] observed, at electron microscope level, biopsies from normal appearing epithelium in patients with squamous cell carcinoma of the upper aero digestive tract and they reported that morphologic abnormalities were consistent with the concept that carcinogenesis is a multistep process of sequential neoplastic development extending over a long period of time [3] (Fig. 1).
Only two years later, in 1984, Strong et al. [4] observed that field cancerization could be demonstrated by supravital staining with toluidine blue or by electron microscopic study of random biopsies taken from apparently normal mucosa [4].
Both authors not only confirmed Slaughter's statements with clinical and morphological means but also identified tobacco and alcohol exposure as the "yet-unknown carcinogenic agent" able to condition mucosal behaviour towards cancer development.

Biomolecular evidences of Slaughter's field cancerization

In 1996 Califano et al.[5], in response to the lack of knowledge surrounding genetic progression of head and neck squamous cell carcinoma and genetic basis for field cancerization, published a PCR based analysis of loss of heterozygosis (LOH) at selective genes putatively involved in head and neck carcinogenesis. They studied a population of eighty-seven lesions of the head and neck, including pre-invasive lesions and benign lesions associated with carcinogen exposure. Observing the level of accumulation of gene losses at different degrees of pre-neoplastic lesions they found that it was possible to identify a model that could explain the progression of a mutated epithelium towards squamous cell carcinoma. However, despite some mutations were more likely to occur at specific stages or pre-malignancy, they suggested that accumulation rather than the order of genetic events led mutated cells in the progression towards cancer.

Fig. 1. Apparently normal oral mucosa can present small areas of altered epithelial keratinocytes.



Genes studied by Califano and colleagues [5] included the 9p21 locus corresponding to an area of genetic loss common to many solid tumors containing p16, a cyclin-dependent kinase inhibitor involved in cell cycle regulation. Region 11q13 that includes the bcl-1/int-2 locus, an amplicon carrying the proto-oncogene cyclin D1, the p53 gene locus located at 17p13, the 3p21 locus and 13q21 locus that contains an area with frequent LOH near the retinoblastoma locus [5].
In particular, 3p and 17p losses were more frequent in mucosa undergoing dysplastic modification while 11q and 13 q losses could be found in epithelium preceding malignant transformation.
Califano's results were confirmed by Patridge et al. in 1997 [6] and Lydiatt in 1998 [7 8] where LOH was found in dysplastic tissue surrounding tumours and histologically normal mucosa respectively.
Genetic approach paved the way to a large number of studies with different molecular techniques that tried to disclose hidden mechanisms of oral carcinogenesis with the aim at discovering clinically relevant biomarkers to be used in clinical practice.
As a consequence, the extension of the field and the related premalignant lesion whence the field could have been arisen, became a major concern.
Noteworthy, Ai [9] in 1999 detected by FISH chromosome aneuploidy in mucosa distant from the carcinoma. Interestingly, 9/10 tested patients were smokers with respect to only one non-smoker patient with aneuploidy. Molecular tests can be of difficult application in daily practice, therefore several authors tested immunohistochemical markers useful to evidence areas od filed cancerization.
Van Oijen e Slootweg in 2000 [10] demonstrated that immunostaining of P53 and EGFR was abnormal in apparently normal mucosa of smokers when compared to non-smokers subjects (Fig. 2).
Tabor et al. [11] demonstrated that the presence of Ki67 positive mature keratinocytes, corresponded to areas of LOH. This observation has been validated by Montebugnoli et al. [12-14] who demonstrated that the immunohistochemical analysis of ki67 in oral mucosa located in the cheek opposite to the OSCC can act as prognostic biomarker as, when overexpressed, had an impact on the aggressiveness of the primary tumour.
On the other hand, studies speculated on how far the field could extend. Interestingly Griffioen GH, et al. [15]. In 2015 reported that patients successfully treated for head and neck cancer died of primary lung cancers suggesting that the field could extend not only through the entire upper aero digestive tract, but also in the deep respiratory system (Fig. 3).

Multiple oral lesions: different models for field cancerization and the problem of clonality

The theory of field cancerization derives from the effort of explaining the increased occurrence of local second-

Fig. 2. Immunohistochemical staining for TP53 in a restricted "patch" of oral epithelium supports the theory according to which the progression of a field of genetically altered kerationcytes precedes and promotes oral carcinogenesis.

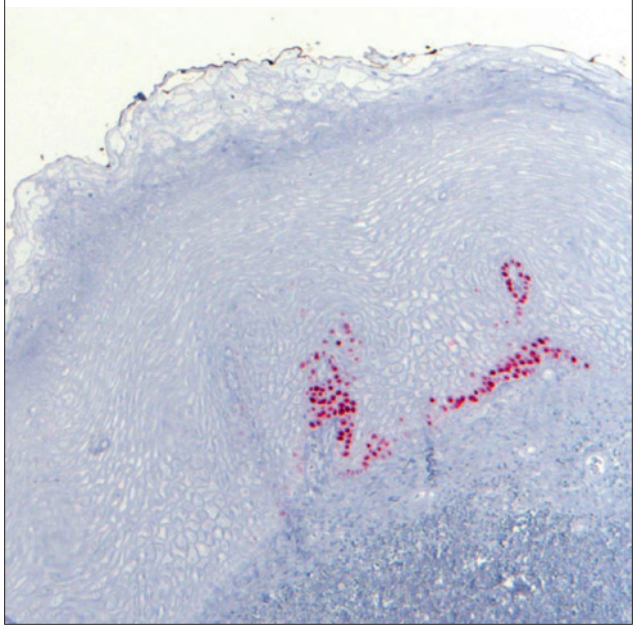
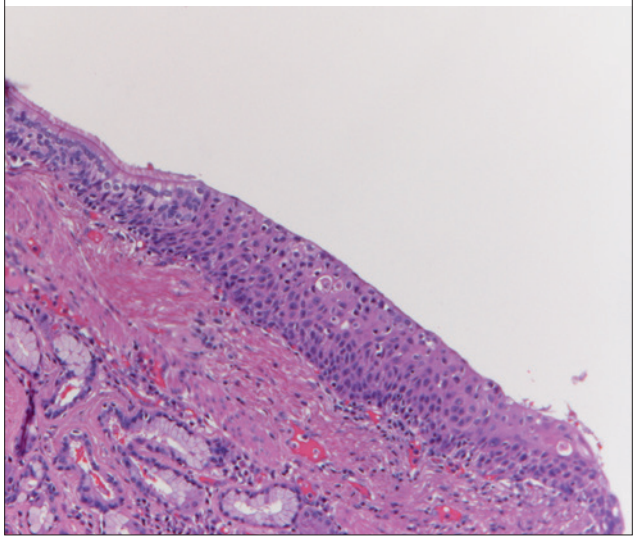


Fig. 3. Smokers can present small areas of dysplasia in bronchial epithelium.



ary tumours in oral cavity and upper aero digestive tract. However, the exact mechanisms through which this phenomenon occurs are still matters of debate and different theories has been formulated so far.

In 1999 Garcia et al. [16] described the presence of cluster of cells usually positive for *TP53* in the normal mucosa of patients with head and neck squamous cell carcinoma. He called these clusters "patches".

Assuming a monoclonal origin for oral squamous carcinoma, he hypothesized that these clusters of genetically altered cells could clonally expand as a consequence of proliferative advantages over non mutated cells generating greater area of mutated mucosa, better defined as "field". Hence, according to Califano's model of carcinogenesis [17], the accumulation in the field of further mutations could lead to the development of independent neoplastic events.

On the other hand, an alternative theory reviewed by van Oijen et al. in 2000 [10], reported that the occurrence of multiple pre(neoplastic) events could also result from a migration of mutated cells that, acting as micro metastasis, could generate different tumours at different local sites.

Apart from field cancerization theoretical models, the distinction between a local recurrence (LR) a metastasis or a second primary tumour (SPT) always represented a critical clinical issue able to deeply influence therapy and prognosis.

Before genetic approach, Second Primary Tumours (SPT) were usually defined by many researchers using Warren and Gates [18] criteria developed in 1932. This method was based on clinical and morphological parameters. In particular, SPTs were defined as two neoplastic lesions showing definitive and distinct pictures of malignancies after excluding that one could be the metastasis of the other. However, in case of two lesions both in the same anatomical area, the minimal distance to exclude a local recurrence was controversial as some researchers accepted 2 cm while others 1.5 cm.

Furthermore, second events may chronologically develop synchronously or methacronously depending on whether the interval between the two carcinomas is lower or higher than six months respectively. Similarly, to spatial criteria, also time of occurrence could easily be confounding in the diagnosis of a second event.

As aforementioned criteria were easily matter of debate and scarcely took into account the theory of field cancerization, in 2002 Braakhuis [19], proposed a modification based on genetic profiles.

Evidence deriving from many works on LOH and *TP53* mutations led Braakhuis to identify four types of second events: Local recurrence, metastasis, second primary tumours and second field tumours.

In particular, common molecular profiles between the primary tumours and the second lesions should be interpreted as consistent with local recurrence or metastasis depending if the second event developed in adjacent or distant site respectively. On the other hand, partially different genetic profile between the two lesions, should be suggestive of a secondary field tumour that, arising from a preconditioned field but followed different carcinogenetic pathways. Second Primary Tumours, being genetically and clinically independent should instead show different genetic profiles.

Further studies [20-23] confirmed the utility of Braakhius modified criteria and disclosed new genetic methodologies to perform clonal analysis of multiple squamous cell carcinomas.

Remarkably, the high frequency rate of mtDNA mutations in tumours, especially those found in the D-loop

region, a non-coding region, along with numerous mitochondrial genomes present in a single cell, has made mtDNA a reliable marker for clonality assays from microdissected paraffin-embedded tissue samples [24 25].
As a result, mtDNA analysis not only demonstrated higher diagnostic sensibility when compared to clinical and temporal criteria, but also resulted more informative with respect to *TP53* analysis, reflecting the ability of some squamous cell carcinoma to follow carcinogenic pathways different from p53 mutations.
In addition, mtDNA analysis resulted highly useful in disclosing possible mechanisms behind the cancerization of skin graft after surgery such as the spread of the clonal cell population to the cutaneous flap stimulated by cytokines produced by the grafted skin.
In fact, as reported by Foschini et al. [20], in all three studied cases, the neoplastic lesions arising in the skin graft showed a clonal relationship with the previous OSCC and, on the basis of the results obtained by mtDNA analysis, could be considered as a recurrence of the primary OSCC.

HPV and field cancerization

The assumption that infection with high-risk human papillomavirus (HPV) could etiologically be linked to the development of HNSCCs, particularly those carcinomas that arise in the oropharyngeal region, is now universally accepted and supported by scientific evidence.
HPV-positive oropharyngeal squamous cell carcinomas (OPSCCs) are characterized by an epidemiologic, demographic and clinical profile that deviates from the profile of conventional HPV-negative OPSCCs. Lower number of local recurrences, and of second primary tumours have been reported.
Whether this better clinical behaviour reflects or not the absence of a "field effect" in HPV-positive OPSCCs it's still debated as results from researches are conflicting.
In 2013 Joseph et al. [26] published the analysis of 4 patients with tonsillar HPV-positive OPSCC who developed synchronous or metachronous second lesions in the contralateral tonsil and found that the HPV DNA sequences derived from the index tumor and corresponding SPT were 100% concordant, indicating that the index and SPTs were caused by the same HPV-16 variant. They also suggested three different models that could explain the "field effect" in the setting of virus-related cancers. According to their findings, second primary tumours caused by the same HPV-16 variant could reflect chronic exposure to an external reservoir. Alternatively, the initial site of HPV inoculation and cancer development could itself serve as an internal reservoir of HPV particles for subsequent infections and subsequent cancer development or could act as epicentre for clonal expansion and migration of HPV-infected cells to other sites.
Conversely, in 2014 Rietbergen et al. [27] studied 152 HPV-positive OPSCC, and analyzed at Next generation sequencing (NGS), PCR and immunohistochemistry the respective surgical margins and SPTs.
Interestingly they found that with respect to surgical margins, only those who contained tumour resulted positive for the same HPV strain while all tumor- negative resection margins scored negative for HPV. Moreover, as far as SPTs analysis was concerned, they only found one patient with HPV-positive metachronous SPT in the head and neck region, which was clonally related to the index tumor.
Their results seem to suggest that that transcriptionally active HPV virus seems to be absent in the mucosa surrounding an HPV-positive OPSCC.

Future perspectives

Despite field cancerization concept is now widely accepted, we are far from understanding entirely the biology of squamous cell carcinoma.
Indeed, In the last decades studies deriving from the relationships between cancer and inflammation, the discovery of epigentic and new theoretical models of cancer biology such as cancer stem cells or tumour heterogeneity have disclosed new potential mechanisms and pathways of carcinogenesis that will eventually have a significant impact on both research and clinical practice.
Of particular interest, Mignogna et al. [28], reported that patients affected by Oral Lichen Planus, a chronic autoimmune potentially malignant disorder of the oral cavity, have increased risk of developing multiple oral squamous cell carcinoma, suggesting that in the theory of field cancerization, chronic inflammation should be taken into account as carcinogenic-conditioning agent.
Consequently, they speculate that the activated inflammatory cells and cytokine network may act as tumorigenesis promoters, and, in the same manner, influence clonal spreading and supporting the process of field cancerization in these chronic inflammatory disorders.
These results suggest that protocols aimed at monitoring fields of altered mucosa should be performed far earlier than cancer development in order to intercept malignancies and relate second lesions at initial stages.
The term "epigenetics" refers to heritable but revertible changes in gene expression that do not involve changes in the underlying DNA sequence. Epigenetic alterations include methylation of CpG dinucleotides and changes in chromatin structure that may cause silencing of tumor suppressor genes while other epigenetic changes such as histone acetylation may lead to activation of oncogenes. Epigenetic alterations are common in human carcinomas.
Evidence that noncancerous oesophageal mucosae of ESCC patients exhibit higher levels of *LINE-1* methylation than similar tissues healthy donors, suggests a phenomenon of epigenetic field effect of the ESCC [29].
Similarly Morandi et al. [30] in 2015 demonstrated by non invasive methods that DNA methylation analysis of *GP1BB* and *ZAP70* genes seem to be a promising marker for early detection of OSCC and potentially lesions,

suggesting that an epigenetical pattern of tumorigenesis acts before cancer development.

Interestingly, a better understanding of epigenetic field defect in oesophageal mucosae will provide improved opportunities for cancer risk assessment, diagnosis and treatment options.

Cancer stem cells (CSC) model for carcinogenesis assume the existence of a pluripotent sub-population of cells in the tumour that have properties of self-renewal, tumor initiation, migration and metastasis. This model has gained emerging interest in the last decade but evidence of this particular mechanism in oral squamous cell carcinoma is still controversial.

As reviewed by Simple in 2015 [31], CSCs markers such as ATR, ABCG1 have been found in the tumor-adjacent normal mucosa providing evidence toward their possible role in field cancerization. In addition, the author describe a hypothetical model though which CSC can initiate and orchestrate oral tumorigenesis. As reported, Cancer stem cell driven field cancerization is initiated by the carcinogen assault leading to a mutation in the stem cell residing in the normal epithelium. Such carcinogenic hit transform the stem cell into cancer stem cell which will proliferate and initially form a patch of transit amplifying cell (TAC), eventually expanding into a field. Accumulation of mutagenic hit in these cells of the field will form the primary tumor. According to cancer stem cells properties, The CSCs of the field will also migrate laterally to spread the field and form genetically similar tumours. This hypothesis is confirmed by the appearance of squamous cell carcinoma, genetically related with the primary OSCC, appearing on the skin graft used to repair the surgical defect [20].

Albeit interesting, further studies on CSC model for oral field cancerization are needed to clarify the exact role and therapeutic potential.

In conclusion, since the publication of Slaughter's first description of field cancerization in 1953 our perspective of head and neck squamous cell carcinoma has progressively improved and yet Slaughter's intuition never suffered attacks or critics. On the contrary, every new discovery as well as each new technological improvement always confirmed the theory of field cancerization by adding new important insights into a highly complex disease. We hope that in the future we will be able to fully understand the biology of oral squamous cell carcinomas and hamper the development of multiple secondary lesions whose observations led Slaughter to formulate his statements and still represent one of the most critical issue of Head and neck squamous cell carcinoma.

References

[1] Slaughter DP, Southwick HW, Smejkal W. *Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin*. Cancer 1953;6:963-8.

[2] Roth D, Sage HH. *Defective photochemical repair in epithelium predisposed to field cancerization*. Cancer 1969;24:511-9.

[3] Incze J, Vaughan CWJr, Lui P, et al. *Premalignant changes in normal appearing epithelium in patients with squamous cell carcinoma of the upper aerodigestive tract*. Am J Surg 1982;144:401-5.

[4] Strong MS, Incze J, Vaughan CW. *Field cancerization in the aerodigestive tract-its etiology, manifestation, and significance*. J Otolaryngol 1984;13:1-6.

[5] Califano J, van der Riet P, Westra W, et al. *Genetic progression model for head and neck cancer: implications for field cancerization*. Cancer Res 1996;56:2488-92.

[6] Partridge M, Emilion G, Pateromichelakis S, et al. *Field cancerisation of the oral cavity: comparison of the spectrum of molecular alterations in cases presenting with both dysplastic and malignant lesions*. Oral Oncol 1997;33:332-7.

[7] Lydiatt WM, Anderson PE, Bazzana T, et al. *Molecular support for field cancerization in the head and neck*. Cancer 1998;82:1376-80.

[8] Lydiatt WM, Davidson BJ, Schantz SP, et al. *9p21 deletion correlates with recurrence in head and neck cancer*. Head Neck 1998;20:113-8.

[9] Ai H, Barrera JE, Pan Z, et al. *Identification of individuals at high risk for head and neck carcinogenesis using chromosome aneuploidy detected by fluorescence in situ hybridization*. Mutat Res 1999;439:223-32.

[10] van Oijen MG, Slootweg PJ. *Oral field cancerization: carcinogen-induced independent events or micrometastatic deposits?* Cancer Epidemiol Biomarkers Prev 2000;9:249-56.

[11] Tabor MP, Braakhuis BJ, van der Wal JE, et al. *Comparative molecular and histological grading of epithelial dysplasia of the oral cavity and the oropharynx*. J Pathol 2003;199:354-60.

[12] Montebugnoli L, Badiali G, Marchetti C, et al. *Prognostic value of Ki67 from clinically and histologically 'normal' distant mucosa in patients surgically treated for oral squamous cell carcinoma: a prospective study*. Int J Oral Maxillofac Surg 2009;38:1165-72.

[13] Del Corso G, Tardio ML, Gissi DB, et al. *Ki-67 and p53 expression in ghost cell odontogenic carcinoma: a case report and literature review*. Oral Maxillofac Surg 2015;19:85-9.

[14] Montebugnoli L, Frini F, Gissi DB, et al. *Histological and immunohistochemical evaluation of new epithelium after removal of oral leukoplakia with Nd:YAG laser treatment*. Lasers Med Sci 2012;27:205-10.

[15] Griffioen GH, Louie AV, de Bree R, et al. *Second primary lung cancers following a diagnosis of primary head and neck cancer*. Lung Cancer 2015;88:94-9.

[16] Garcia SB, Park HS, Novelli M, et al. *Field cancerization, clonality, and epithelial stem cells: the spread of mutated clones in epithelial sheets*. J Pathol 1999;187:61-81.

[17] Califano J, Westra WH, Meininger G, et al. *Genetic progression and clonal relationship of recurrent premalignant head and neck lesions*. Clin Cancer Res 2000;6:347-52.

[18] S Warren OG. *Multiple primary malignant tumors: a survey of the literature and a statistical study*. Am J Cancer 1932;16:1358-414.

[19] Braakhuis BJ, Tabor MP, Leemans CR, et al. *Second primary tumors and field cancerization in oral and oropharyngeal cancer: molecular techniques provide new insights and definitions*. Head Neck 2002;24:198-206.

[20] Foschini MP, Morandi L, Marchetti C, et al. *Cancerization of cutaneous flap reconstruction for oral squamous cell carcinoma: report of three cases studied with the mtDNA D-loop sequence analysis*. Histopathology 2011;58:361-7.

[21] Tarsitano A, Leonardi E, Morandi L, et al. *Late skip lymph node metastasis of oral squamous cell carcinoma or metastasis of unknown second primary tumor? Answer by mitochondrial DNA analysis*. Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:e11-4.

[22] Montebugnoli L, Leonardi E, Morandi L, et al. *Genetic relationship between multiple squamous cell carcinomas arising in the oral cavity*. Head Neck 2014;36:94-100.

[23] Morandi L, Tarsitano A, Gissi D, et al. *Clonality analysis in primary oral squamous cell carcinoma and related lymph-node metastasis revealed by TP53 and mitochondrial DNA next generation sequencing analysis*. J Craniomaxillofac Surg 2015;43:208-13.

[24] Morandi L, Pession A, Marucci GL, et al. *Intraepidermal cells of Paget's carcinoma of the breast can be genetically different from those of the underlying carcinoma*. Hum Pathol 2003;34:1321-30.

[25] Morandi L, Marucci G, Foschini MP, et al. *Genetic similarities and differences between lobular in situ neoplasia (LN) and invasive lobular carcinoma of the breast*. Virchows Arch 2006;449:14-23.

[26] Joseph AW, Ogawa T, Bishop JA, et al. *Molecular etiology of second primary tumors in contralateral tonsils of human papillomavirus-associated index tonsillar carcinomas*. Oral Oncol 2013;49:244-8.

[27] Rietbergen MM, Braakhuis BJ, Moukhtari N, et al. *No evidence for active human papillomavirus (HPV) in fields surrounding HPV-positive oropharyngeal tumors*. J Oral Pathol Med 2014;43:137-42.

[28] Mignogna MD, Fedele S, Lo Russo L, et al. *Field cancerization in oral lichen planus*. Eur J Surg Oncol 2007;33:383-9.

[29] Matsuda Y, Yamashita S, Lee YC, et al. *Hypomethylation of Alu repetitive elements in esophageal mucosa, and its potential contribution to the epigenetic field for cancerization*. Cancer Causes Control 2012;23:865-73.

[30] Morandi L, Gissi D, Tarsitano A, et al. *DNA methylation analysis by bisulfite next-generation sequencing for early detection of oral squamous cell carcinoma and high-grade squamous intraepithelial lesion from oral brushing*. J Craniomaxillofac Surg 2015;43:1494-500.

[31] Simple M, Suresh A, Das D, et al. *Cancer stem cells and field cancerization of oral squamous cell carcinoma*. Oral Oncol 2015;51:643-51.