Oral field cancerization: an update on current concepts

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Abstract

There always exists a field with genetically altered cells with a high risk of developing premalignant and malignant lesions. It may often happen that an individual stem cell is genetically altered and can cause the formation of a clone or a patch which is likely to turn into a tumor. This explains the higher recurrence rates following tumor resections. It is essential to identify and to treat this field in order to have greater chances to prevent cancer and achieve a better outcome. This article reports concepts, theories and markers for the assessment of field cancerization.

Introduction

The oral cavity is one of the predominant and prevalent sites of development of potential malignancies, since it comes into direct contact with many carcinogens. The squamous cell carcinoma is one of the most common malignancies developed in the oral cavity with an average survival rate of about 5 years.[1] Despite monitoring the original tumor site following an advanced surgical and non-surgical therapy, the overall mortality rate remains unchanged probably due to the recurrence of the tumor either locally or at a remote site.[2] The devel-

opment of recurrences and second primary tumors, even when surgical margins are histopathologically tumor-free corroborates the concept of field cancerization.[3]

Field cancerization also called *field defect* or *field effect* is a well-known process of transformation of an existing precancerous lesion into a malignancy.[4] Oral field cancerization implies that oral cancer does not arise as an isolated cellular phenomenon, but rather as an anaplastic tendency involving many cells at once that results into a multifocal development process of cancer at various rates within the entire field in response to a carcinogen, such as in particular tobacco.[5] This definition is often used to describe the development of abnormal tissues around a tumorigenic area, resulting into an oral multifocal cancer in individual sites, which later coalesce and create atypical areas, even after complete surgical removal. This may explain the cause for second primary tumors and recurrences.[6]

Prolonged exposure to carcinogens alters the state of the epithelium, making it susceptible to developing a multifocal carcinoma, which can also derive from independent mutations in the absence of any genetic influence. Multifocal areas of precancerous alterations may trigger this process without involving in particular an individual cell which becomes malignant.[7] This process may explain the high recurrence rate of carcinomas even after the patient undergoes surgery and radiation therapy. Tumor recurrence is most often due to changes in the preconditioned epithelium, now more prone to cancer, which is located next to the suture line or has healed over the site of a tumor eliminated by radiation therapy.[7]

Criteria used to diagnose multiple carcinomas

Warren and Gates initially formulated a set of criteria to diagnose multiple primary carcinomas which were modified later by Hong *et al*.[8,9] The criteria to be met are as follows: i) the neoplasm must be distinct and anatomically separate. A multi-centric primary neoplasm is diagnosed when a dysplastic mucosa is present next to it; ii) a potential second primary carcinoma which represents a metastasis or a local relapse should be excluded. It has to occur 3 years after the initial diagnosis or it should be separate from the first tumor by at least 2 cm from the normal epithelium.

Numerous factors determine the progression of a field into a new tumor and must therefore be accurately reviewed and followed up. A pre-malignant field often requires a much longer period of approximately 67-96 months to progress into an invasive carcinoma.[10,11]

History of field cancerization

The concept and the definition of field cancerization was first introduced by Slaughter *et al*. in 1953, when he analyzed the tissues adjacent to

squamous cell carcinoma.[12] The concept was first examined in the aerodigestive tract, where multiple primary tumors and local recurrent tumors originate from the anaplastic tendency of multiple cells. The term *lateral cancerization* was coined later to suggest the lateral spread of tumors, which occurs due to a progressive transformation of the tissue adjacent to the tumor rather than the expansion of pre-existing cancer cells into the adjacent tissue.[13] On the basis of a broad analysis of 783 carcinoma patients, Slaughter *et al*. observed that the entire epithelium adjacent to the tumor exhibited more than one independent area of malignancy Later, the expression of *field cancerization* was adopted, as these findings suggested that the exposure to carcinogen-induced mucosal changes makes the adjacent area susceptible to multiple malignant foci. The concept of field cancerization was extended to other organs, including oropharynx, esophagus, lungs, stomach, colon, cervix, anus, skin and bladder.[14]

The oral cavity was proven to be most susceptible to this process, as it is exposed to a wide range of environmental carcinogens which affect the entire mucosa and result into the simultaneous occurrence of premalignant states. This led to various molecular analyses to investigate the genetic mutations and clonality to validate this carcinogenesis model.[14] In particular these findings were reported in 1950's when the Watson and Crick model was first described. Later numerous molecular techniques provided unequivocal evidence supporting the concepts proposed by Slaughter *et al*.[6]

Concept of field cancerization

Field cancerization involves the formation of multiple patches of premalignant disease with a higher-than-expected rate of multiple local second primary tumors.[15] In the oral cavity, tobacco and alcohol act in synergy as primary carcinogens in the development of squamous cell carcinomas. The environmental carcinogens reach simultaneously a large area and can damage a large proportion of cells contributing to premalignant states within the entire surface exposed.[14]

The process of carcinogenesis initiates from multiple genetic and epigenetic alterations in the mucosa which can lead to the clonal expansion of premalignant daughter cells in a particular field.[16] The genetically altered stem cells form a clonal unit comprising daughter cells from which the patch expands into the adjacent areas in subsequent steps following further modifications.[14] This triggers sequential cellular transformations that ultimately lead to the replacement of the normal epithelium by a proliferating field (Figure 1). However, there is a population of cells with early genetic changes, which does not demonstrate any histological alterations, thus explaining the concept of field cancerization.[12]

Patches: field precursor lesions

In the epithelium, there is a cluster of cells with cancer-related genetic alterations which can be demonstrated by TP53 immunostaining.[17] These clusters were named *patches* by Garcia *et al*. and were considered equivalent to a clone or a clonal unit.[18] They were defined as a small group of cells which share a contiguous common genotype at the time of observation.[18] These patches are usually positive for TP53 in the normal mucosa of patients with head and neck squamous cell carcinoma and are frequent in multiple primary head and neck tumors.[19,20] These units with transit stem cells and amplifying cells which undergo differentiation make up the squamous epithelium. When a stem cell develops a genetic alteration, the cells derived from it continue to carry the same clonal patch resulting in the formation of a cluster containing TP53 immunopositive cells.[18]

Theories of field cancerization

Three theories have been postulated to explain the occurrence of carcinomas in specific sites (Figure 2). One theory states that multiple squamous cell lesions occur independently of each other. This is due to the exposure of the oral cavity to carcinogens in at the same time leading to multiple genetic abnormalities in the entire area.[12] An alternative theory states that multiple lesions arise due to the migration of dysplastic and altered cells with two different patterns,[21,22] as follows: i) migration of malignant cells through the saliva (micro metastasis); ii) intra-epithelial migration of the progeny of initially transformed malignant cells. This is different from the metastasis, since malignant cells are usually encountered by the lymph nodes and blood where they first develop.

There are two methods of investigation to assess these theories.[23] The first method considers the alterations in the tumor adjacent mucosa in the histologically normal tumor adjacent mucosa in smokers and drinkers of alcoholic beverages and in the normal tumor adjacent mucosa of non-smokers and non-drinkers.[24] The tumor adjacent mucosa in head and neck squamous cell carcinoma smokers and non-smokers shows migrating tumor cells and therefore similar alterations. However these cells are usually absent in healthy smokers. Furthermore, the presence of migrating cells in tumor-adjacent mucosa (TAM) in advanced tumors are usually identical to the alterations in
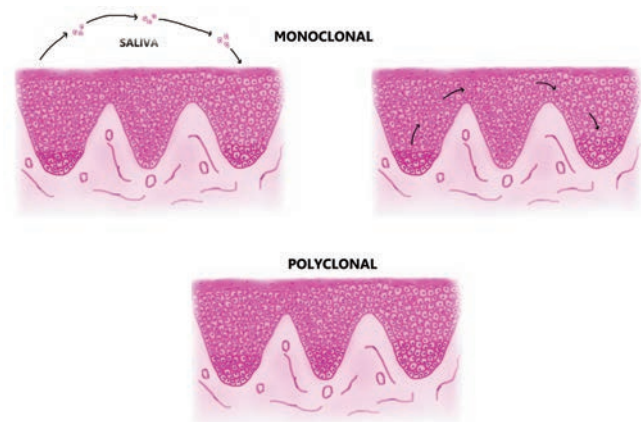

Figure 1. Field cancerization model: evolution of a normal epithelium to a patch, a field and a fully invasive carcinomatous lesion.
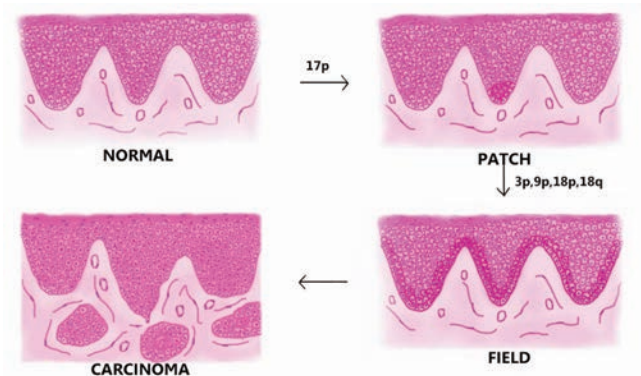

Figure 2. Theories of field cancerization.

the primary tumor, whereas the presence of migrating progenitor cells in TAMs suggests that at least a few early tumorigenic alterations are identical in the tumor adjacent mucosa and in the invasive tumor. In a few smoking head and neck squamous cell carcinoma patients, there are no migrating cells, thus suggesting that they are smoking-induced independent events. Similarly there are also alterations in the tumor adjacent mucosa in head and neck carcinoma patients which are present in the normal mucosa of healthy smokers.[24,25]

The second method of investigation is based on the clonality of the multiple malignant and premalignant lesions by determining genetic alterations in head and neck squamous cell carcinoma patients.[22,26] Two separate lesions are said to develop from a single clone when they share common genetic alterations. This clonal relationship between several premalignant and malignant lesions suggests that the tumor cells or the progenitor cells drift and result in cancerization.[27] However in the absence of a clonal relationship between multiple lesions, it is more likely that they derive from an independent event.[28]

Field cancerization model

The process of carcinogenesis begins with a stem cell which develops one or more genetic and epigenetic alterations. Subsequently a clone of genetically altered cells forms a patch or a cluster. As a result of further genetic alterations, the stem cell escapes the normal growth control pattern and gains advantage by developing into an expanding clone. Later the lesions progress and become a field which displaces laterally the normal epithelium. The field, having a genetically altered clonal unit, has an enhanced proliferative activity which is the driving force of the entire process.[26.]As the lesions grow in size, additional genetic hits arise in the region resulting in various sub-clones within the field. The clones diverge at different times creating a relatively large number of altered stem cells due to clonal divergence and selection. However they share the same clonal origin. Eventually this process ends up in the formation of an invasive cancer. The probability of developing cancer from a genetically altered stem cell depends on the nature of the affected stem cell itself and of additional hits. The carcinogenesis model we propose is therefore based on a monoclonal origin and includes three main steps:[29]

- First phase (patch formation): conversion of a single stem cell (patch) into a group of cells (clone) which carry the genetic alterations without a proper growth control pattern.
- Second phase (clonal expansion): additional genetic alterations develop and the patch proliferates taking advantage of its enhanced growth potential and forms a field which displaces the normal epithelium.
- Third phase (transition to tumor): the clone or field eventually turns into an overt carcinoma with invasive growth and metastasis.

Molecular concepts of field cancerization

The expression of various markers in the epithelium and connective tissue components can help determine the field cancerization (Table 1).[1,17,24,29-67] Molecular findings indicate the presence of cytokeratin 7, 8, 13, 16 and 19 at abnormal sites and abnormal levels within the epithelium.[56,68] Also well-defined foci of cyclin D1 expression are present in the normal mucosa adjacent to the carcinomatous areas.[29] Several studies have shown a rise in the levels of epidermal growth fac-

tor receptor in the tumor-associated normal mucosa.[25,40,41,44,69-72] A five-fold increase in the levels of messenger ribonucleic acid (mRNA) of transforming growth factor was observed.[44] Increased levels of proliferating epithelial cells were demonstrated using the proliferating cell nuclear antigen and the argyrophilic nucleolar organizer region (AgNOR).[58,73] A rise in Ki-67 expression was also observed and suggests an increased number of proliferating cells.[59]

Also marked variations in the expression of enzymes were detected in the epithelium. The expression of isoenzyme glutathione S-transferase was found to be significantly higher in the supra-basal and superficial layers of the normal oral mucosa in head and neck carcinoma patients. In the literature also an increase in the detoxification enzymes was reported, which is intriguing, since they protect against carcinogenic attacks.[62]

Vascular markers like VwF and CD31 have also proved to be particularly high in the normal mucosa adjacent to carcinomatous areas due to the upregulation of angiogenic stimulators like the vascular endothelial growth factor or in association with the downregulation of angiogenic inhibitors.[45] The most promising marker of field cancerization is p53 which shows a strong positive correlation with the progression of the tumor from a benign to a malignant state.[30]

Concepts of field cancerization based on clonality models

Oral field cancerization occurs by either cell migration or development from independent cells. If multiple tumors occur from the migration of cells from their primary source, the genetic alterations of the primary cell are carried over to all the progenitor cells. However in case of independent cells, the process is different. The assessment of a clonal marker based on the early identification of genetic events is important to investigate the development of the primary lesion and its progression through the expansion of cells.[25] The method used initially was the X chromosome inactivation which occurred when large patches of cells were derived from a common ancestor especially during embryonic development.[74] Later, karyotyping was used in the metaphase stage to compare their appearance and detect ploidy and chromosomal breaks.[75] Further 6 microsatellite assays with markers like 3p, 8p, 9p, 13q, and Rb were performed. The detection of mitochondrial DNA mutations was also performed. Currently p53 mutations are used as clonal markers for multiple primary tumors, as their expression has been observed in the normal tissue far from the tumor sites.[76]

Chromosomal aberrations in the field

A trend towards anuesomies of chromosomes 2, 6 and Y were observed in the normal mucosa of smokers. Polysomies of chromosomes 7 and 17 has also been reported in distant tumor sites along with a loss in chromosome Y. Allelic loss of chromosome 13q14 has also been detected using the jicrosatellite analysis.[77] This suggests that allelic loss can precede the histological changes in head and neck cancer. Metastatic tumors demonstrate an overpresentation of chromosomes 5p, 6p and 7p. Nodal involvements are characterized by a deletion on chromosome 7q, 10q, 11p, 11q, 15q and 20p and an over representation of chromosomes 19q and 20q. This molecular analysis predicts the metastatic tendency in head and neck squamous cell carcinoma patients.[78]

Clinical implications and consequences

It is often noticed that a tumor arises from a site where a surgical excision of the tumor was performed in the same anatomic area. This kind of recurrence from a site where surgery was performed to remove completely a tumor explains the concept of field cancerization.[79,80] The presence of genetically altered cells in a particular field acts as a risk factor for cancerization and has important consequences.[26] The presence of pre-neoplastic cells in larger numbers in a proliferating field is likely to be associated with a high risk of malignant transformation.[16] The probability of developing a second primary tumor in a patient with a history of previous squamous cell carcinoma is around 20%.[81]

The detection of this field which is prone to the development of cancer is based on the identification of molecular signatures in a genetically transformed, yet histologically normal field called peri-tumoral cancer field. This relies on tumor markers, which are specific for the tumor. Hence the identification of these reliable tumor biomarkers will help monitor the progression of the tumor, thus preventing the transformation of pre-malignant lesions into an invasive cancer.[82] To date,

Table 1. Markers in the determination of field cancerization.

Categories	Field Cancerization Marker	References
Specific genomic markers	Tumor suppressor/oncogenes or cell cycle control genes	Houten et al.[17]
	p53	El-Naggar[30]
		Franklin et al.[31]
	p16	Pannone et al.[32]
	Cyclin D1	Izzo et al.,[29] Bartkova[33]
	P21(WAF1/CIP1)	El-Gazzar et al.[34]
	Retinoblastoma gene (rb)	Patrick et al.[35]
	C-jun	Hu et al.[36]
	3p (unidentified)	El Naggar et al.[37]
	Proto oncogene alterations	Ortiz et al.[38]
	Ras (H, K, N ras)	Merrick et al.[39]
	ErbB1	Werkmeister et al.[40]
Growth factors/receptors	EGF/EGFR	Szyma ska et al.[41]
	VEGF	Astekar et al.[42]
	CD34	Lopez Blanc et al.[43]
	TGF-α	Grandis et al.[44]
Vascular markers	VwF	El-Gazzar[45]
	CD31	El-Gazzar[45]
	αVβ3	El-Gazzar[45]
	α-SMA	El-Gazzar[45]
Genomic markers	Genetic studies chromosomal anomalies/ aberrations	
	Loss of heterozygosity	Califano et al.[46]
	DNA sequence analysis	Silveira et al.[1]
	Gene profiling	Haaland et al.[47]
	Mitochondrial genome changes	Dakubo et al.[48]
	Nuclear aberrations	
	Micronuclei	Bloching et al.[49]
Indices for genomic instability	Aneuploidy	Ai et al.[50]
	Microsatellite markers	Partridge et al.[51]
	DNA adducts	Wiencke et al.[52]
Squamous differentiation antigens	Cytokeratins - 7, 8, 13, 16, and 19	Copper et al.[53]
		Ogden et al.[54]
		Bonger et al.[55]
		Bosch et al.[56]
	Secretory products – ABH antigen	Bonger et al.[55]
	Telomerase	Heaphy et al.[57]
Proliferation indices	Nuclear antigens	Shin et al.[58]
	PCNA	Vanoijen et al.[24]
	Ki-67	Kamel et al.[59]
	Thymidine labelling index	Lopez Blanc et al.[43]
	AgNOR	
Nuclear retinoid receptors	Retinoic acid receptors	Smith et al.[60]
	Retinoid X receptors	Fischer et al.[61]
Oxidative stress	Glutathione S transferase	Bongers et al.[62]
	Superoxide dismutase	Ahmed et al.[63]
	Heat shock proteins	Kaur et al.[64]
Apoptosis	Bcl2, Bax	Birchall et al.[65]
	Chromatin condensation factor	Cherkezyan et al.[66]
	Caspase	Bascones-Martinez et al.[67]

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several tumor biomarkers have been reported in various types of cancer, including cancer in the head and neck region,[6] lungs,[31] colon, rectum,[83,84] breast,[85] stomach,[86] prostrate[87,88] and bladder.[89] Several markers have been used to analyze the molecular aspects of the tumor-adjacent normal tissue and surgical margins to determine the presence of field lesions. The markers commonly used are loss of heterozygosity, microsatellite alterations, chromosomal instability, mutations in the *p53* gene, which are generally detected by polymerase chain reaction, immunohistochemistry and *in situ* hybridization.[90]

Understanding the terminology

The definition of second primary tumor is exclusive intended for second tumors which arise independently from the first tumor. However, when the history shows the occurrence of a second tumor arising from the same field, it is always preferable to use the definition of second field tumor (SFT). It is important to mark this difference, since clinical consequences can vary with differing etiologies. It is also to be noted that third and fourth field tumors arise from secondary field tumors. Hence a routine follow-up is mandatory in cases of SFT. The definition of local recurrence applies to lesions arising from the remaining tumor cells and local residues of the field which develop into cancer. Hence, a local recurrence is also a form of SFT.[91]

Conclusions

The definition of field cancerization refers to a group of genetically altered clones of cells in multifocal patches, which are prone to the development of synchronous and metachronous tumors. The field cancerization theory also emphasizes the high probability of recurrences in patients with head and neck squamous cell carcinoma. Therefore a frequent oral examination with histological studies and molecular testing are mandatory for patients after surgery, especially for those at high risk of developing malignancies. Though numerous markers have been identified to help determine the field effect, the entire process is still controversial, therefore further investigations are still in progress to gain a better understanding of carcinogenesis and to use the biomarkers foreseen in this concept for cancer prevention purposes.

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