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Review Article

Field Cancerisation in Oral Cavity: Recent Concepts and Review with Special Reference to Cancer Stem Cells

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Abstract

The concept of field cancerisation was conceived by Danley. P. Slaughter and co-workers in 1953. Oral field cancerisation can be defined as the presence of one or more areas consisting of epithelial cells that have cancer-associated genetic or epigenetic alterations. Rather than local recurrences, development of second primary tumours (SPT) strengthens the concept of field cancerisation. The occurrence of multiple tumours can be substantiated by 2 competing hypothesis: Polyclonal theory/Classic theory, Monoclonal theory/Alternative theory. The clinical implication of field cancerisation lies in the identification of peri-tumoral cancer field which deceives a pathologist by their naïve histology. These peri-tumoral cancer fields can be sculptured out by the presence of certain molecular markers. Cancer stem cells (CSC) are a small subset of cells which were found to be highly tumorigenic with capability of self-renewal and behaviour akin to tumour progenitor cells. Thus, an altered field is the forerunner of a full-blown carcinoma and detecting these areas through routine histology and molecular analysis is of utmost importance in patients especially in post treatment phase which may spare the patient of mortality and morbidity of advanced cancer treatments.

Keywords: Field Cancerisation; Second Primary Tumors; Polyclonal and Monoclonal Theories; Oral Cavity; Markers in Field Cancerisation; Cancer Stem Cells

Abbreviations

SPT: Second Primary Tumor; SFT: Second Field Tumor; LOH: Loss of Heterozygosity; CSC: Cancer Stem Cells; NSC: Normal Stem Cells; EMT: Epithelial-Mesenchymal Transition

Introduction

Oral cancer is a major cause for morbidity and mortality worldwide. In a survey conducted by Global Oral Cancer Forum in 2016 the incidence of lip and oral cavity cancers was an astounding 300,000 cases approximately annually. Oral cancer is particularly dangerous because in its early stages it may not be noticed by the patient, as it can frequently prosper without producing pain or symptoms they might readily recognize, and because it has a high risk of producing second primary tumours (SPT). This means that patients who survive a first encounter with the disease have up to a 20 times higher risk of developing a second cancer. The concept of field cancerisation becomes relevant in this context.

The concept of field cancerisation was conceived by Danley. P. Slaughter and co-workers in 1953 to account for the development of multiple primary tumours and local recurrences in the aero digestive tract [1]. On the basis of recent genomic and proteomic studies, oral field cancerisation can be defined as the presence of one or more areas consisting of epithelial cells that have cancer-associated genetic or epigenetic alterations [2]. The apparently normal looking mucosa adjacent to the area of tumour may harbour cells which carry mutations that can pave way to the development of second primary tumours despite the complete resection of the primary tumour.

Rather than local recurrences, development of second primary tumours strengthen the concept of field cancerisation as local recurrences can develop due to incomplete resection, while development of SPT cannot be attributed to iatrogenic error.

How is Second Primary Tumor Different from Local Recurrences?

According to clinical criteria local recurrence is defined as cancer that develops from same place of the primary tumour or occurring at a distance < 2 cm from the initial tumour and within 3 years after the primary tumour [3].

SPT on the other hand is diagnosed based on Warren and Gates criteria of 1932 [4]. The criteria states:

1. Histological confirmation of malignancy in both the index and secondary tumours.
2. There should be at least 2 cm of normal mucosa between the tumours. If the tumours are in the same location, then they should be separated in time by at least three years.
3. Probability of one being the metastasis of the other must be excluded.

Later Cunliffe., *et al.* sub classified SPTs as synchronous and metachronous SPTs [5]. Synchronous SPT developed immediately or within 6 months of the initial diagnosis whereas second carcinoma found after 6 months of primary lesion is termed metachronous SPT.

The diagnosis of local recurrence and SPT is essentially from a clinical perspective. There existed confusion between these and another term, Second field tumour (SFT). Hence Braakuhis., *et al.* in 2003 proposed classifying SPT into SFT(Second Field Tumour) and True SPT [6]. SFT is a tumour that has developed from the same field as the index tumour and share identical genetic pattern as the primary tumour. Whereas true SPT is an independently evolved carcinoma with unrelated genetic changes.

The occurrence of multiple tumours can be substantiated by 2 competing hypothesis [7]:

- Polyclonal theory/Classic theory
- Monoclonal theory/Alternative theory

Polyclonal theory

Exposure of oral cavity to carcinogens leads to multiple genetic abnormalities which are independent of each other. This in turn leads to development of multiple primary tumours of diverse clonality.

Monoclonal theory

A single genetically altered cell through mucosal spread may give rise to multiple tumours. These tumours have a common clonal origin. Two migratory patterns attributed are:

a) Micro metastasis through saliva
b) Intraepithelial migration of the progeny of pioneer mutated cell

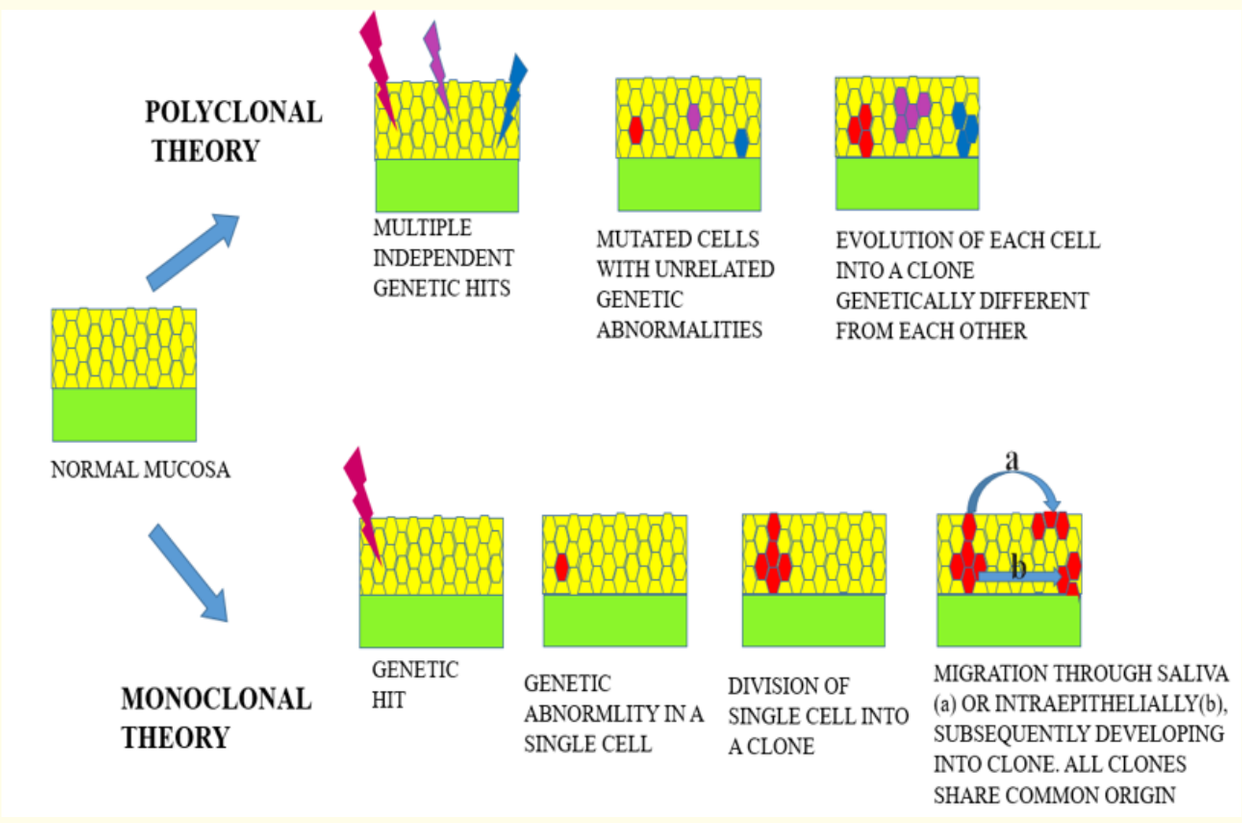

Figure 1: Clonality of multiple tumors [1,2].

Evolution from Normal Epithelium to Abnormal Epithelium

There are 3 critical steps involved

1. **Patch formation:** In epithelium clusters of cells with cancer related genetic alterations were named as patches by Gracia., *et al* [8]. Patch can be defined as a small group of cells which share a contiguous common genotype at the time of observation [7]. Cells of the patch mainly shows TP53 mutation. There are few data concerning the patch size in human tissues. Patch size in the oral epithelium is derived as a maximum diameter of 200 TP53 immunopositive cells and a cell diameter of 10 μm [9].
2. **Clonal expansion:** Patch acquires more genetic alterations leading to uncontrolled growth giving rise to a clone. Expansion of clone displaces the adjacent normal tissue. As the lesion becomes larger, additional genetic hits give rise to various sub clones within the field. Different clones diverge at a certain time point with respect to genetic alterations but do share a common clonal origin [3,7].
3. **Transition to tumour:** The process of clonal divergence and selection eventually results in a sub clone evolving into invasive carcinoma [4,7].

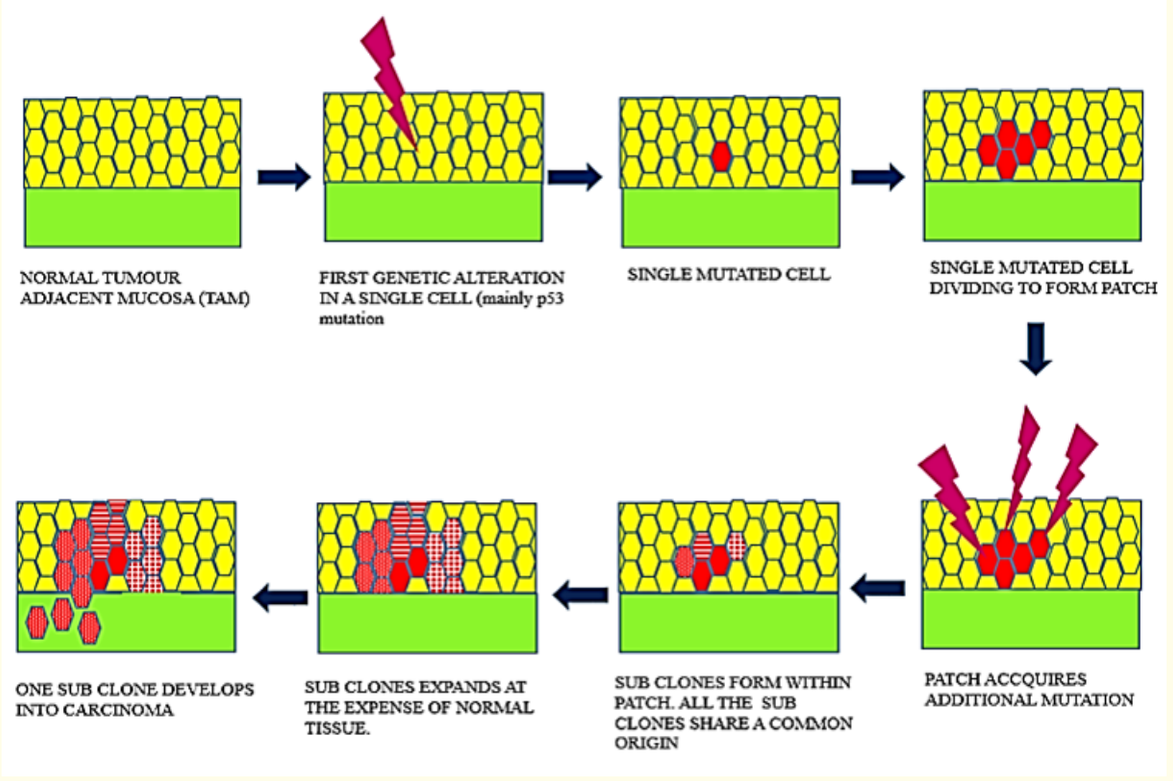

Figure 2: Evolution from normal to abnormal.

Molecular Perspective of Field Cancerisation

The clinical implication of field cancerisation lies in the identification of peri-tumoral cancer field which deceives a pathologist by their naïve histology. These peri-tumoral cancer fields can be sculptured out by the presence of certain molecular markers. To establish clonal relationship between multiple tumours, clonal markers are required [5,10].

To qualify as a good marker the following criteria has to be fulfilled [10]:

(1) Occur very early in the development of the primary lesion
(2) Be maintained during progression of the lesion
(3) Exhibit sufficient variability
(4) Be applicable in majority of lesions

The genomic aberrations commonly encountered are loss of heterozygosity (LOH), microsatellite instability, chromosomal alterations, mutations in the p53 gene, which are generally detected by polymerase chain reaction, Immunohistochemistry and in situ hybridization [7].

Although the concept of field cancerisation was proposed by Slaughter after analysing cases of squamous cell carcinoma in aerodigestive tract, it was later adopted to other body parts and tissues including oral cavity. The concepts and theories of field effect could be applied very well to the oral cavity. The genetically altered field adjacent to area of neoplasm could be implicated in recurrences even after resection of oral carcinomas with sufficient resection margins. A number of studies were undertaken by many researchers using an array of markers to determine the field effect in oral cavity and most of them have emerged with promising results. Some of the markers to determine field effect in oral cavity are mentioned in the table 1

Marker	Expression/Function in Normal Oral Mucosa	Expression in Altered Oral Field
p53 [11,12]	Tumour suppressor gene regulating cell cycle progression, DNA repair, cellular senescence and apoptosis	Expansion of multiple clones of mutant p53-containing cells
Cyclin D1[2,13,14]	Participates in the regulation of the phosphorylation status of the retinoblastoma protein (pRb) and is thought to play a role in driving cells through the restriction point in late G1	Early dysregulation of cyclin D1 expression
Retinoblastoma gene [15,16]	Tumour suppressor-responsible for a major G1 checkpoint, blocking S-phase entry and cell growth	Phosphorylated form of retinoblastoma is present in the carcinoma as well as in adjacent mucosa
Bcl-2 expression [17]	Majorly apoptosis inhibitor	Lack of bcl-2 expression
EGFR [16,18]	Induces cell proliferation or differentiation	Dramatic increase of EGFR levels
VEGF expression and sub epithelial vascularisation [19,20]	VEGF- in physiological and also in most pathological angiogenesis	Borders adjacent to carcinomas exhibit an increase in VEGF expression and sub epithelial vascularisation.
TGF-α [17,20]	TGF-α is ligand of EGFR	mRNA level of TGF-α was 5-fold increase in normal TAM compared with mRNA levels in control normal mucosa
Cytokeratin [21-23]	*CK 8*: No immunoreactions in the oral mucosa *CK 19* positive expression throughout the basal cell layer Hyper proliferative epithelia are known to express 16	Cytokeratin 8 over expressed no expression of CK 19 in lesional tissue of OSCC, the normal mucosa adjacent to OSCC showed an enhance expression at basal and suprabasal cells Increased expression of CK16
ABH Antigen [24,25]	Type 2 chain ABH antigens are expressed on parabasal layers of normal oral epithelium	Increased expression
PCNA [26,27]	Appears in all proliferating cells-plays an important role in DNA synthesis, DNA repair, cell cycle progression and cell proliferation	PCNA expression was fourfold higher in basal layer and six-fold higher in parabasal layer
Ki-67 [28,29]	Confined to isolated cells/occasional cells adjacent to the basal lamina, i.e., in the basal layer and mostly in the parabasal layer with no positivity in the superficial layer	Significantly higher cell proliferation rate in parabasal layers determined by increased Ki-67 expression
AgNOR [30,31]	AgNOR value - measure of the rate of cell proliferation.	Increased AgNOR expression

Table 1: Marker: Expression in normal oral mucosa and altered oral field [6].

Genetic markers of field cancerisation

Studies done by Califano., et al. showed that areas of apparently benign mucosa adjacent to malignant lesion demonstrates early genetic events, which are derived from a common clone [32]. Researches over the last decades has revealed that field lesions shows a plethora of genetic aberrations including deletion of key chromosomal regions at 3p, 4q, 8p, 9p, 13q and 18q [33], amplification of cyclin genes [34], mutations and LOH affecting P 53 gene [32,35]. Cells with this mutated P53 genes can acquire additional genetic hits easily, such that when a critical threshold of aberrations is reached, cancer results [35].

LOH in field cancerization

Measuring LOH with microsatellite analysis shows that normal mucosa adjacent to tumour or surgical margins have tumour associated genetic alterations [36]. LOH at either 9p21 or 3p21 is seen in both histopathologically early (field) and advanced regions, supporting the role of these loci as important early event in tumour progression [32].

Microsatellite Assays

Allelic alterations at more polymorphic loci within the critical chromosomal regions can be assessed using microsatellite assays and this helps to distinguish a field that harbours genetic aberration from a normal adjacent mucosa or as Sauter., *et al.* so aptly coined the phrase "distinguish our benign pussy-cat from baby tigers" [35]. Study conducted by Mao., *et al.* with two microsatellite markers one at 3p14 and the other at 9p21 demonstrated that the probability of developing a tumour was 45% if allelic alterations at either of these two key chromosomal regions was detected [37].

Cancer Stem Cells in Field Cancerisation

Cancer stem cells (CSC) are a small subset of cells which were found to be highly tumorigenic with capability of self-renewal and behaviour akin to tumour progenitor cells [38]. The evolution of cancer stem cells via genetic and epigenetic changes is responsible for tumorigenesis, inter and intra-tumoral heterogenicity, metastasis and even recurrences.

CSC and Normal stem cell (NSC) share a lot of features like [39]:

1. Capacity for self-renewal
2. Ability to differentiate into multiple progenitor cell types
3. Angiogenic induction
4. Active telomerase expression
5. Increased membrane transporter activity
6. Migratory and metastatic capacity
7. Apoptotic resistance
8. Long life spans

CSC and NSC differ in their regulation of replication. CSC shows unregulated division due to defect in genetic and epigenetic pathways and result in production of mutated daughter cells [40]. The progeny of CSC has limitless survival and proliferative potential and shows more plasticity compared to NSC progeny which ultimately becomes a differentiated cell with limited or no replicative potential.

Origin of CSC is hypothesised to be one of the following

1. A normal tissue-specific stem cell or its progenitor undergoes several genetic as well as epigenetic alterations to give rise to a CSC [41].
2. From a stem cell which has acquired a precancerous phenotype during embryogenesis [41].
3. From mature somatic cells through
    a. Horizontal gene transfer [42]
    b. Induction of genomic instability like aneuploidy [43]
    c. Contributions from microenvironment of cells e.g. IL-6 produced by non-stem cancer cells [44]
    d. De-differentiation [45]
    e. Epithelial- Mesenchymal Transition (EMT) [46]
4. Fusion of cells: Normal stem cells fuse with differentiated cells or tumour cells to form CSC [47]. These fused cells are called heterokaryon.

According to monoclonal theory of altered fields, the mutated cell has to move to distant site within the epithelium. The motility ability is rendered to the transformed cell by loss of E-cadherin mediated adhesion, which is a hallmark of EMT [48]. These transformed cells which has undergone EMT to attain motility also shows properties of CSCs. In addition, 2 subsets of CSC are identified-migratory CSC and non- migratory CSC. These evidences indicate that CSCs are probably the cells with intra-epithelial migratory capability, thereby being the most likely candidates to execute the monoclonal process of field cancerisation.

In the polyclonal process of field cancerisation, the NSCs at different sites in the mucosa, undergo stepwise transformation into CSCs through independent, carcinogen-mediated molecular alterations. These CSCs proliferate leading to the development of clones/patches at different sites. Additional genetic hits give rise to further divergence in the sub-clones within the field [49]. Hence an increase in expression of CSC in tumour adjacent mucosa could be considered as a fore runner of cancer.

There are no universal markers of CSCs because these cells change their phenotypes depending on their microenvironment. Besides, there is overlap of markers between CSC and NSC. The markers of CSC could be grouped as membrane antigens and transcription factors. Membrane antigens include CD133, CD 44, E-Cadherins etc [38]. OCT3/4, NANOG and SOX2 constitute the transcription factors of CSC. Aldehyde dehydrogenases (ALDHs), which include 18 isoenzymes expressed in humans, are more robust markers of CSCs.

Conclusion

When it comes to carcinogenesis known is a drop and unknown is an ocean. Most challenging arena is the early detection of primary or recurrence or a second primary. An altered field is the forerunner of a full-blown carcinoma. Detecting these areas through routine histology and molecular analysis is of utmost importance in patients especially in post treatment phase. Such an approach will spare the patient of mortality and morbidity of advanced cancer treatments. Further exploration of molecular markers and genomic changes and the significance of stem cells are required to strengthen the concept of field cancerisation which helps to bring them as a main stage protocol post therapy.

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