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Review

Cancer stem cells and field cancerization of Oral squamous cell carcinoma

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SUMMARY

Oral squamous cell carcinoma (OSCC) has a high propensity for local failure, which is attributed to recurrence at the primary site or the development of second primary tumors (SPT). Field cancerization that refers to the existence of transformed cells in areas adjacent to the primary tumor, has been attributed to be one of the probable reasons underlying disease relapse. The carcinogenic process necessitates multiple molecular events for the transformation of a normal cell into a cancer cell. This implies that only the long-time residents of the epithelium, such as the stem cells, might be the candidates capable of accumulating these genetic hits. These transformed stem cells- the 'Cancer stem cells' (CSCs), are further known to be equipped with the properties of tumor initiation and migration, both of which are essential for orchestrating field cancerization. The concept that the CSCs might be responsible for field cancerization in OSCC has not been explored extensively. If the role of CSCs as the primary units of field cancerization process is established, their presence in the mucosa adjacent to the tumor may be an indicator for local recurrence and/or development of second primary tumors. In this review, we examine the available evidence in literature exploring the possibilities of CSCs driving the process of field cancerization and thereby being the underlying mechanism for disease recurrence and development of SPT.

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Introduction

Loco-regional recurrence and development of second primary tumors are the major factors impacting the survival rate in head and neck cancer. Despite intense, multi-modal local therapy, recurrence at the primary sites occurs in about 10–30% of the patients while the incidence rate of second primary tumor varies between 2% and 30% [1–3]. 'Field cancerization,' referred to as the occurrence of molecular abnormalities in the tumor adjacent mucosal field, is a concept that attempts to explain local disease failure, both recurrence and occurrence of second primary tumors. In epithelial cancers, tumor adjacent normal mucosa is also exposed to the carcinogen and is reported to develop abnormal molecular changes. The major molecular alterations, considered as the hallmarks of field cancerization, are mutations in oncogenes/tumor suppressor genes, loss of heterozygosity (LOH) and genomic instability. The cells with these alterations are known to gain the capacity to develop and expand the neoplastic field. These mutant, pre-cancerous cells are then hypothesized to replace the normal cells in the mucosa, consequently rendering the epithelia susceptible to further genetic/epigenetic hits, thereby triggering tumor formation [1,4].

Cancer stem cells (CSC) are a pluripotent sub-population of cells in the tumor that have properties of self-renewal, tumor initiation,

Abbreviations: HNSCC, head and neck squamous cell carcinoma; OSCC, Oral squamous cell carcinoma; LOH, Loss of heterozygosity; CSC, Cancer stem cells; CIS, Carcinoma insitu; SPT, second primary tumor; TAC, Transient Amplifying Cells; ALDH1, Aldehyde dehydrogenase1; EMT, Epithelial-to-Mesenchymal Transition; Klf4, Kruppel like factor 4; ABCG2, ATP Binding cassette sub-family G member 2; ESA, Epithelial Specific Antigen; ATR, ATM and Rad-3 related; ANKRD50, Ankyrin Repeat Domin 50; MSC, mesenchymal stem cells; TAF, tumor associated fibroblast; TRbeta1, Thyroid hormone receptor beta 1; FHIT, Fragile histidine triad; FRA3B, Fragile site, aphidicolin type, common, fra(3); ABCC4, ATP-binding cassette, sub-family C (CFTR/MRP), member 4; MMP, Matrix metalloproteinase; VEGF-A, Vascular Endothelial Growth Factor-A; CK-19, Cytokeratin-19; EGFR, Epithelial growth Factor Receptor; NSC, Normal Cancer Stem Cell; MPT, multiple primary tumor; SFT, second field tumor; ATRA, All Trans Retinoic Acid.

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migration and metastasis [5–14], whether these cells can orchestrate the process of field cancerization is a question that has not been addressed in detail. In this review, we have assessed the available evidences towards the role of CSCs in the process of field cancerization and thereby the potential of CSC-specific biomarkers as a predictor of local recurrence and second primary tumor formation.

Field cancerization

Principle

The concept of '*Field cancerization*', coined by Slaughter in 1953, proposes that the normal tissue adjacent to the tumor harbor certain pre-neoplastic genetic finger prints which can eventually lead to development of local recurrence or second primary tumors. Slaughter and his group based this concept on the following observations: (i) tumor adjacent mucosa being molecularly 'abnormal' (ii) multifocal areas of precancerous changes develop due to a prolonged and widespread exposure to carcinogens (iii) oral cancer often consists of multiple independent lesions that sometimes coalesce and (iv) formation of second primary tumors and recurrences can be explained by the presence of residual abnormal tissue after surgery [15,16].

Origin of field cancerization

Cellular basis

The underlying cellular basis of field cancerization is explained by two different models. The '*polyclonal origin*', theory proposes that mutations occur in multiple sites of the epithelium due to continuous carcinogen exposure and thereby lead to multi-focal carcinomas or lesions of independent origin [17]. These tumors arising in adjacent fields are thus genetically different. An alternative theory is the '*monoclonal origin*' of the field wherein the mutant cells from the initial lesion migrate and develop multiple lesions that share a common clonal origin. In order to explain the underlying mechanisms driving this concept, three theories have been postulated. *The first theory* suggests that tumor cells or tumor progenitor cells migrate through the submucosa to another site. *The second theory* implies that cells shed into the lumen of the primary site form tumors at an adjacent secondary site. *The third theory* suggests that the continuous genetically altered fields in the epithelium lead to the development of clonally related neoplastic lesions that develop via lateral spreading in the same or adjacent anatomical areas [18].

Genetic basis

As per the existing genetic progression model of field cancerization, the transformation of a normal epithelium to a cancerous one is a gradual step-wise process. This model of carcinogenesis is primarily based on evidence that correlates genetic alterations with the histological progression of HNSCC [19]. Mutations in TP53 (17p) in a single cell was considered to be the initial step that triggers the process, the mutant cell then proliferates into a clonal unit and then into a patch of mutated cells. *In the next step*, the patch transforms into the field characterized by other subsequent cancer-related genetic alterations in chromosome positions 3p, 9p, 8p and 18q. This field eventually replaces the normal tissue. Subsequent mutations in 11q13 are then suggested to transform the field to a carcinoma *in situ* (CIS) [19].

Califano et al. have also described the genetic progression model of field cancerization in head and neck cancer. According to this model, the transformation of normal mucosa is initiated by hits to the 9p region. The benign hyperplasia that develops as a consequence, further transforms into dysplasia due to successive mutations in the 3p and 17p regions. The modifications in 17p region are suggested to drive the development of the first patch of mutated neoplastic cells. The patch further expands into the field, which then transforms to cancer with mutation in 11q,13q and 14q chromosomes [20].

With increasing evidences pointing to the cancer stem cells (CSC) being a subgroup of cells responsible for tumor initiation and recurrence, the question does arise as to whether the genetic changes characteristic to field cancerization are initiated within the resident stem cells of the mucosa.

Cancer stem cells in field cancerization

Cancer stem cells

Cancer stem cells (CSCs) are a small population of cells within the tumor that are tissue specific, slow dividing and with unlimited self-renewal capacity [21]. The origin of these cells is explained by three different processes. The first model envisaged that a normal, tissue-specific stem cell undergoes several genetic as well as epigenetic alterations to give rise to a CSC [4]. This model gains significance owing to the fact that normal and cancer stem cells share attributes such as self-renewal and drug resistance, though they differ in their deregulated proliferative capacity, invasion and metastatic properties [21,22]. Another school of thought states that CSCs originate from stem cells that acquire a precancerous phenotype during developmental stage itself. [4,23,24] (Fig. 1). The third model, shared by a number of cancer biologists, states that the CSC originate from mature tumor cells that undergo de-differentiation into a stem cell through modifications in signaling pathways and regulatory mechanisms [25–28]. In addition, the de-differentiation of mature oral epithelial cells is also another possible origin of CSCs [4,23,24] (Fig. 1). Independent of the origin of CSCs, evidences do confirm their capacity to initiate and sustain tumor development and cell migration; properties which may support the view that CSCs are probably the underlying tools of field cancerization.

Cancer stem cells in initiation of oral carcinogenesis

Oral carcinogenesis necessitates a multi-step process leading to the transformation of a normal cell into a cancer cell [20,29,30]. In oral mucosa, wherein the differentiated epithelial cells have a high renewal rate (14 days) [31], it is likely that normal stem cells (NSCs), the long-term residents of epithelium have a greater chance to accumulate the necessary genetic hits required for malignant transformation. The NSCs, after acquiring one or more of the genetic mutations, attain a growth advantage and subsequently develop into a patch/lesion constituted of a cluster of cells called the Transient Amplifying Cells [18,32]. Evidence for this hypothesis can also be drawn from the recent observation that long-term nicotine exposure of oral mucosa can lead to up regulation of the CSC marker ALDH1A1, suggesting a possible induction of CSC behavior due to carcinogen assault [33].

An alternative de-differentiation theory of CSC origin has been proposed in epithelial malignancies. It has been shown that transfection of Oct4 and Sox2 in pancreatic cancer and melanoma cells leads to an enrichment of the CSC content. This process is known to be mediated by NANOG and KLF4, molecules that regulate cell cycle and Epithelial Mesenchymal Transition (EMT) [25,26]. Nanog has been further implicated in de-differentiation of p53 deficient astrocytes into brain CSC-like cells [27] while mutant p53 in itself, is known to be a de-differentiating factor in human osteosarcoma cells [28]. These results provide evidences toward the concept of a de-differentiation-mediated CSC origin.