Cancer stem cells and field cancerization of head and neck cancer - An update

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ABSTRACT

Oral cancer results due to multiple genetic alterations that transform the normal cells in the oral cavity into neoplastic cells. These genetic changes in a particular tumor field lead to a rapid expansion of preneoplastic daughter cells producing malignant phenotype but the malignancy results due to such genetic changes occurr over several years. The morphological changes in these transformed cells help in the diagnosis of malignancy. Thus, the early changes at the gene level are present in the population of daughter cells in the organ, which explains the concept of field cancerization. Cancer stem cells (CSCs) represent a group of cells that have the capacity of self-renewal and have the potential to differentiate into other types of tumor cells. This review explains the cellular and genetic basis of field cancerization and the role of cancer stem cells in field cancerization.

Keywords: Cancer stem cells, field cancerization, tumor

Introduction

The targeting genetic changes that are responsible for the malignant conversion of normal cells occur over several years. This emphasizes the importance of the treatment of early malignancies to prevent carcinogenesis.[1,2]

The term "field cancerization" was introduced by Slaughter in1953.[3] The term "lateral cancerization" indicates the lateral spread of the tumor as a result of alterations of cells adjacent to a tumor, rather than the spread and destruction of the adjacent epithelium by preexisting cancer cells.[4] Later, Slaughter and colleagues used the term "field cancerization" to describe early genetic changes in the epithelium resulting from carcinogens that lead to the development of multifocal tumors.[5]

"Oral field cancerization" refers to genetic changes that occur in the oral mucosa adjacent to the tumor site, and it explains the failure of disease, both recurrence and occurrence of second primary tumors. In oral squamous cell carcinoma, the sites adjacent to the normal mucosa are also exposed to the mutagens and thus develop abnormal genetic 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. These cells

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with genetic changes gain the ability to develop and expand the neoplastic field. The normal mucosal cells in the oral cavity are thus replaced by these mutant precancerous cells consequently rendering the epithelia susceptible to further genetic/epigenetic hits, thereby triggering tumor formation.[6,7]

The "field cancerization" concept, states that the normal tissue adjacent to the tumor harbor certain preneoplastic genetic fingerprints, which can eventually lead to the development of local recurrence or second primary tumors.

Slaughter and his group based this concept on the following observations:[3]
(i) Tumor adjacent mucosa being molecularly "abnormal"
(ii) Multifocal areas of precancerous changes develop due to prolonged and widespread exposure to carcinogens
(iii) Oral cancer often consists of multiple independent lesions that sometimes coalesce
(iv) Formation of second primary tumors and recurrences can be explained by the presence of residual abnormal tissue after surgery.

Origin of Field Cancerization

Cellular basis

The underlying cellular basis of field cancerization is explained by two different models. The "polyclonal origin", the 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.[7] 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. To explain the underlying mechanisms driving this concept, three theories have been postulated.

1. The first theory suggests that tumor cells or tumor progenitor cells migrate through the submucosa to another site.
2. The second theory implies that cells shed into the lumen of the primary site form tumors at an adjacent secondary site.
3. 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.[8]

Genetic basis

As per the existing genetic progression model of field cancerization, the transformation of 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 oral squamous cell carcinoma (OSCC).[9] 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 into carcinoma *in situ* (CIS).[9]

Molecular Basis of Field Formation

Epigenetics field and DNA methylation in field cancerization

Epigenetic changes are seen in both cancerous and noncancerous tissue. Epigenetic information is defined as information other than the DNA sequence that is faithfully replicated upon somatic cell replication. It is carried by DNA methylation at CpG sites, histone modifications, and polycomb complex formation.[10] DNA methylation is an epigenetic alteration that occurs in cancer. In cancer cells, "genome-overall hypomethylation and regional hypermethylation" are present. The hypomethylation can lead to genomic instability and is considered to be involved in tumor progression.[11,12]

Genetic markers for altered field

The identification of the peritumoral cancer field is a challenge for a pathologist. These peritumoral cancer fields can be distinguished by the application of certain molecular markers. Different clonal markers can be used that have the following characteristics:[13,14]
1. The molecular markers must be applicable in most of the pathologies
2. These must be readily available
3. These must be maintained during the progression of the lesion
4. Exhibit variability

To evaluate the extent of field lesion molecular analyses have been performed on the adjacent normal tissue and margins of the related tumor sites. Various molecular markers can be used such as loss of heterozygosity (LOH), microsatellite alterations, chromosomal instability, and mutations in the *TP53* gene detected by DNA amplification techniques, immunohistochemistry, and *in situ* hybridization.[15-17]

Field precancerization in oral squamous cell carcinoma (OSCC)

Oral squamous cell carcinoma (OSCC) originates from the single precursor cell that undergoes malignant transformation and clonal expansion thus producing monoclonal cancer cell population. The precursor cells that give rise to OSCC may arise either from the tissue-specific stem cells undergoing epigenetic or genetic alterations or from the mature keratinocytes undergoing cytogenetic and epigenetic alterations resulting in its dedifferentiation into an immature progenitor/stem cell that causes dysregulated intracellular pathways. This transformation of mature keratinocytes due to epigenetic and

cytogenetic changes affects the cell cycle progression, DNA repair mechanisms, differentiation, and apoptosis. Further genetic alterations, transform these precancerous keratinocytes into a cancerous phenotype that results in a growing dominance over the normal neighboring cells. These cells undergo clonal expansion and divergence giving rise to a clone of cells having growth advantage over normal neighboring keratinocytes. This produces a precancerized field in the epithelium.[18]

The transformed keratinocytes within the field of precancerized epithelium may sometimes give rise to the second carcinoma. The cells of the new carcinoma and the original carcinoma may have common genetic changes as both of these originate from a proliferating monoclone within the precancerized field.[9]

Cancer stem cells (CSCs) and field cancerization

Cancer stem cells (CSCs) refer to the population of cells that have the capability of self-renewal, metastatic potential, increased apoptotic resistance, and have tumorigenic behavior similar to the tumor progenitor cells. CSCs have defective genetic and epigenetic pathways thus undergo unregulated cell division to produce mutated daughter cells. The evolution of cancer stem cells via genetic and epigenetic changes are responsible for tumorigenesis, inter and intratumoral heterogenicity, metastasis, and even recurrences.[14]

The transformed stem cells or the CSCs have the properties of tumor initiation and progression, both of which are essential for orchestrating field cancerization. These CSCs might be responsible for field cancerization in OSCC.

Origin of CSCs

CSCs are the tumor imitating cells that have the capability of proliferation, differentiation, and self-renew. CSCs play a critical role in tumor imitation, progression, and metastasis. Various factors contribute to the development of the CSCs: Mutation in stem cells/differentiated cells/progenitor cells, genetic imbalance, and cellular microenvironment.[19]

Several hypotheses that explain the origin of CSCs include:
1. Cell fusion
2. Horizontal gene transfers
3. Genetic instability
4. Influence of cell microenvironment

Cell fusion

Cell fusion often leads to the development of cancer and its progression. Generally, a normal stem cell fuses with the transformed cell to produce either a mononucleated (synkaryon) or a multinucleated cell (heterokaryon). The heterokaryon is considered as an intermediate of the synkaryon with chromosome loss. The fusion of cells contributes to the development of cancer and its progression. The hybrid metastatic cells are produced by the fusion of tumor cells with lymphocytes.

Horizontal gene transfer

Horizontal gene transfer produces CSC in three steps: Transferring DNA fragment to the recipient cells, incorporation the transferred genetic sequences into the recipient's genome, and expression of the incorporated genes by the recipient.

Mutations in the somatic cells result in their programmed cell death (apoptosis) and DNA fragmentation. These DNA fragments are taken up by the other somatic cells by the process of phagocytosis or endocytosis resulting in the formation of aggressive cancer stem cells. This process is often termed as horizontal gene transfer.

Genomic instability

Genetic alterations at the chromosomal or the molecular level lead to the instability among the cells and are the fundamental basis of cell transformation and cancer initiation. Genomic alterations in the form of aneuploidy or point mutations often lead to an imbalance in chromosome number and loss of heterozygosity (LOH). The LOH of tumor suppressor genes increases the susceptibility of cells to mutagens and increases the potential of tumorigenesis.[19]

Microenvironment

Cell microenvironment triggers the selective clonal expansion of CSC that originates from the chromosomal mutation in differentiated/progenitor and stem cells. The host microenvironment regulates stem cell differentiation and proliferation and is regulated by various factors like inflammation, infection, and cell injury. The inflammatory microenvironment triggers the formation and clonal selection of cancer stem cells. Injury or infections may induce inflammation responses. Stem cells that reside in the specific tissue may proliferate and repair the tissue injury, but inflammatory cytokines and microenvironment may deregulate the normal stem cells into cancer stem cells. The inflammatory environment may also dedifferentiate cancer cells into cancer stem cells.[19]

A model of field cancerization orchestrated by the CSC was proposed by Jinqiu Feng *et al.*, and it was suggested that the identification of CSC-specific markers proved to be useful in providing novel targets for the prevention and treatment of field cancerization. The expression of ALDH1 and Bmi1 within a single potentially malignant OE lesion significantly correlated with subsequently developing multiple and multifocal carcinomas, which parallels the process of oral field cancerization. Thus, it was suggested that ALDH1 and Bmi1 are well-defined markers of CSC for head and neck cancer.[20]

MPTs (Multiple primary tumors) and polyclonality

Most studies that used clonal markers to investigate the relationship between MPTs or to investigate dysplastic lesions that were remote from each other showed polyclonality. Only a limited amount of MPTs showed the same genetic alterations as evidenced by showing identical microsatellite

alterations, LOH patterns, or cytogenetic features. However, the overwhelming majorities of remote MPTs show no clonal relationships and can, therefore, be assumed to have developed independently. OSCC or adjacent premalignant lesions that are located very close to each other more often show identical genetic changes.[21]

Field Precursor Lesions: Patches

Sometimes clusters of cells with cancer-associated genetic alterations can be found in the epithelium that is much smaller. Concerning tumor-adjacent oral mucosa, clusters (<200 cells diameter) can be observed with TP53 immunostaining.[22,23]

Sequence analysis showed that the type of mutation in *TP53* in these clusters always differed from that in the tumor. These clusters are known as "patches," are defined as a group of cells that share a common genotype, contiguous at the moment of consideration.[24]

Second primary tumor (SPT) and local recurrence

Besides the clinical problems related to the index tumor, head and neck squamous cell carcinoma (HNSCC) patients are at high risk for developing SPTs, often located at the same or an adjacent site. According to the criteria of Warren and Gates,[25] SPT can be defined as:

(a) Tumor presenting a definite picture of malignancy.
(b) A characteristically distinct tumor.
(c) Exclusion of metastatic tumors.

The distance of approximately 2 cm between the first and SPT must be there to exclude the chances of local recurrence.[26,27]

An additional criterion of an SPT at the same or an adjacent anatomical site is that it should occur at least 3 years after the diagnosis of the primary tumor. SPTs can be divided into two groups:

1. Synchronous SPTs: These tumors develop simultaneously with or within 6 months after the initial tumor.
2. Metachronous SPTs: These tumors develop >6 months after the origin of the initial tumor. Most of the SPTs are metachronous originating after the treatment of the initial tumor during the follow-up of cancer patients.[28]

Molecular studies have revealed a new system of a classification method for second primary tumors. Earlier before the application of molecular techniques, these lesions were distinguished based on arbitrary distance and the time to recurrence. If a tumor recurred at the same anatomic site, then some investigators believed that, for it to be considered a second primary tumor, at least three years had to have elapsed between detection of the tumors. These somewhat arbitrary distinctions have been refined by molecular techniques that can identify relationships between lesions. Therefore, the authors suggest a different designation-"

second field tumors" (SFT)-for those lesions that are anatomically distinct but demonstrate genetic similarities.[29]

For those tumors that arise in the same anatomic location postresection, SFTs can be identified as well. Thus, true second primaries would be those lesions that did not share any genetic similarity and therefore likely arose as a result of independent events.[23]

The implication for primary care

Oral field cancerization poses a greater challenge to an oral pathologist as a genetically altered field is the forerunner of oral carcinoma. The presence of a field with genetically altered cells is a risk factor for cancer. Routine histopathology and molecular analysis techniques aid in detecting these areas in patients especially in the posttreatment phase. Such an approach will spare the patient of mortality and morbidity of advanced cancer treatments. To manage the patients of oral field cancerization an oral pathologist must have a detailed knowledge of field cancer.

Oral pathologist play has an important role in early detection of the field areas by using new diagnostic molecular markers, modalities to prevent its progression, and finally, prevent the development of the second primary tumor. Finally, not only early detection and management of oral cancer are important but also equally important are early identification and management of a field to have profound implications on cancer prevention and outcome of the treatment.[12,30,31]

Conclusion

Field cancerization is a well-known and well-documented process of malignant transformation. Several studies confirm the importance of this phenomenon in tumor development. The presence of a field with genetically altered cells is a risk factor for cancer. The cancer risk increases due to the presence of preneoplastic daughter cells in the altered field. This also explains the high incidence of secondary cancers after surgery of the initial carcinoma.

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Conflicts of interest

There are no conflicts of interest.

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