Review Article

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ORAL FIELD CARCINOGENESIS: AN UPDATE

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ABSTRACT:

Patients with head and neck squamous cell carcinoma (HNSCC) often develop multiple (pre) malignant lesions. This finding led to the field of the cancerization theory, which hypothesizes that the entire epithelial surface of the upper aerodigestive tract has an increased risk for the development of (pre) malignant lesions, because of multiple genetic abnormalities in the whole tissue region. Demonstration of alterations in histologically normal tumor-adjacent mucosa from HNSCC patients supported this hypothesis. Currently, the question has been raised whether multiple lesions develop independently from each other or from migrated malignant or progenitor cells. Moreover, almost all primary remote tumors from HNSCC patients appear to be clonally unrelated. Therefore, there is more evidence that field cancerization is due to multiple independent events than to migration of genetically altered cells.

Keywords: Oral squamous cell carcinoma, intra epithelial migration, second field tumours.

INTRODUCTION:

Squamous cell carcinoma is the sixth most common malignancy in men and accounts for approximately 5% of the malignant tumors in the population of developed countries. However, in parts of Southeast Asia, head and neck cancer is the most common malignancy, accounting for up to 50% of the malignant tumors.[1]

Survival of squamous cell carcinoma patients depends on the tumor size, nodal stage, and the success of initial treatment, which has not improved very much during the last decade.[2] In general, a five year survival rate of 50% can be obtained, although some anatomical sites are associated with a less favourable prognosis than others.[3] The prognosis of squamous cell carcinoma patients is adversely influenced by the development of a new tumor, which may arise as a recurrence of an incompletely resected index tumor or may be a second field tumor (SFT) or a second primary tumor (SPT) that has arisen on a genetically altered premalignant field.[4]

These findings led to the field of a cancerization theory, which hypothesizes that the entire epithelial surface of the upper aerodigestive tract has an increased risk for the development of (pre) malignant lesions, because of multiple genetic abnormalities in the whole tissue region.

ORAL FIELD CANCERIZATION

The concept of the field effect in cancer is also known as field defect/field carcinogenesis/condemned mucosal syndrome or field cancerization.[5] Field

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cancerization is a well known and well documented process of malignant transformation. The term 'field cancerization' was proposed by Slaughter et al., in 1953, when studying oral cancer.[6]

On the basis of recent molecular findings, the following definition of field cancerization has been proposed: 'The presence of one or more areas consisting of epithelial cells that have genetic alterations. A field lesion (or 'field' in short) has a monoclonal origin, and does not show invasive growth or metastatic behaviour, the hallmark criteria of cancer.'

A field lesion is preneoplastic; it may have histological aberrations characteristic of dysplasia. The term 'lateral cancerization' was subsequently used to indicate that the lateral spread of tumors was due to a progressive transformation of cells adjacent to a tumor, rather than the spread and destruction of the adjacent epithelium by the pre existing cancer cells.[7]

Organ systems in which field cancerization has been described are: HNSCC in the oral cavity, oropharynx, and larynx; lung; esophagus; vulva; cervix; colon; breast; bladder; and skin.[8]

DIFFERENT THEORIES OF ORAL FIELD CANCERIZATION

The mucosal changes in the entire upper aerodigestive tract (UADT) were generally considered to be the result of exposure to carcinogens that caused multiple genetic abnormalities in the whole tissue region. The occurrence of multiple tumors can be explained by two competing hypotheses.[9]

1. Monoclonal theory (classical view) in which a single cell is transformed, and through mucosal spread, gives rise to multiple genetically related tumors.

2. Polyclonal theory (clonal theory) in which multiple transforming events gives rise to genetically unrelated multiple tumors.

3. An alternative theory for the occurrence of multiple (pre) malignant lesions has been proposed and is based on the premise that any transforming event is rare and that multiple lesions arise due to the widespread migration of transformed cells through the whole aerodigestive tract.[10]

Two types of migration are involved in the concept of this theory:

a. Migration of tumor cells by, for example, saliva (micro metastases)

b. Intraepithelial migration of the progeny of the initially transformed cells.

Apart from the polyclonal or monoclonal concept, a third concept is that a tumor may have a paracrine effect on the adjacent oral mucosa. Tumors have been found to secrete tumor inhibitory factors that include inhibitors of neovascularization (endostatin) as well as promoters of apoptosis. Removal of the primary tumor will remove these

inhibitors of cancer development, and hence, promote second primary tumor formation.[10]

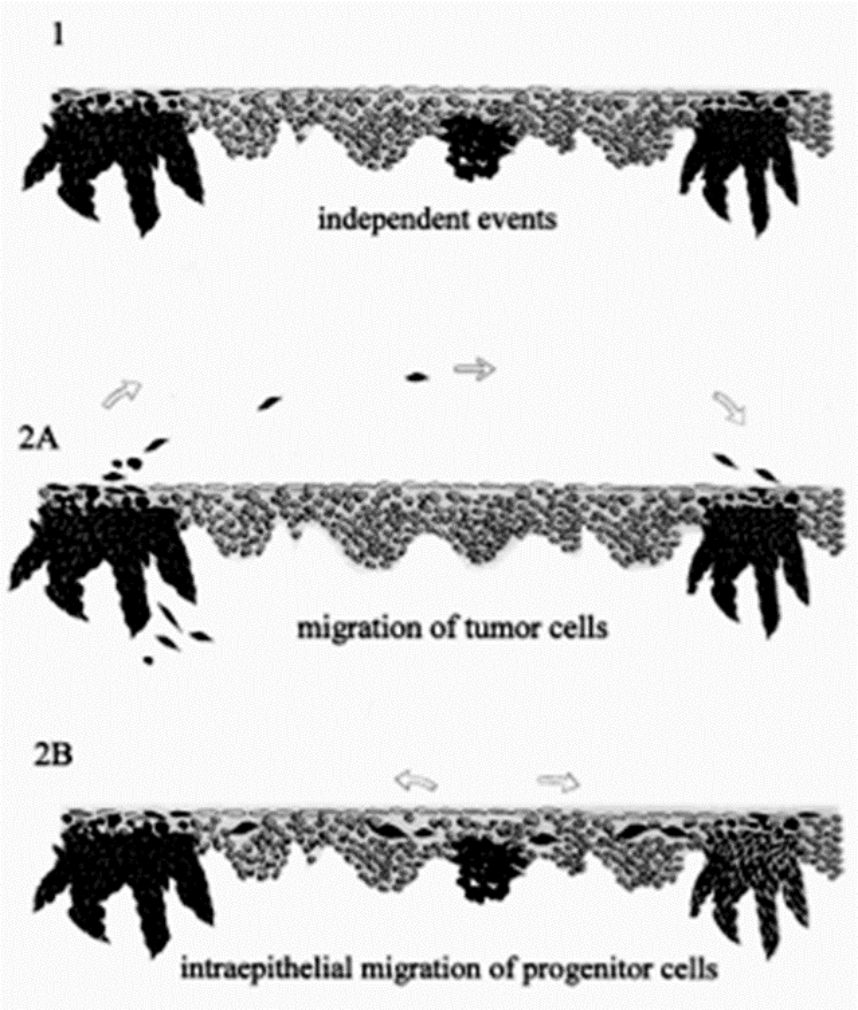

Figure 1: Different field cancerization theories.

FIELD PRECURSOR LESIONS:

Patches in various epithelia, clusters of cells with cancer associated genetic alterations can be found, which are much smaller than the fields are called field precursor lesions.[11]

DEFINITION OF SECOND PRIMARY TUMOR AND SECOND FIELD TUMOR SECOND PRIMARY TUMORS

Besides the clinical problems related to the index tumor, HNSCC patients are at a high risk for developing SPTs, often located at the same or an adjacent site. For a definition of SPT, most clinicians currently use the criteria of Warren and Gates, which were published in 1932:

a. Each of the tumors must present a definite picture of malignancy

b. Each of the tumors must be distinct

c. The probability of one being a metastasis of the other must be excluded.[12]

Histological examination will often find that a tumor is malignant, but with this method, it is difficult to prove that the lesions are distinct. To exclude the possibility of a local recurrence, most studies use a distance of at least 2 cm between the first tumor and the SPT.[13]

An additional criterion of an SPT, at the same or an adjacent anatomical site, is that it should occur at least three years after the diagnosis of the primary tumor. SPTs can be divided into two groups: Synchronous SPTs, which develop simultaneously with or within six months after the index tumor, and Metachronous SPTs, which develop more than six months after the initial tumor. Most SPTs are metachronous and develop during follow up of HNSCC patients, after curative treatment of the first tumor. The term SPT suggests that these tumors and the index tumors have developed independently. Recently, however, genetic studies have shown that, in a proportion of cases, the first and second tumors have originated from the same precursor cell.[14] A new classification method for second primary tumors has been proposed, to account for the information gained from molecular studies.[13]

In the past, these lesions were distinguished as being distinct simply by an arbitrary distance, often 1.5 or 2.0 cm apart. The tumors were also classified by 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 – SFT – for those lesions that are anatomically distinct, but demonstrate genetic similarities.[15]

For those tumors that arise in the same anatomic location post resection, SFTs can be identified as well. Thus, true second primaries will be those lesions that do not share any genetic similarity, and therefore, likely rise as a result of independent events.[15]

FIELD AND SECOND FIELD TUMORS

Fields with genetically altered cells can be large (up to 7 cm in diameter) and are not visible to the treating physician. These facts explain how a field can often be left behind when an HNSCC is resected. The presence of a field with genetically altered cells is likely to be a continuous risk factor for another carcinoma. Indeed, evidence is available to show that cancer has developed from fields that remain in patients after surgery of the initial carcinoma.[16]

Based on the etiology, there are two types of SPTs: One group originates from the same field in which the first primary tumor developed and the second group

has an independent origin. Because the difference in etiology has clinical implications between these two types of SPTs, the SFT is defined as a tumor that has developed from the same field as the index tumor and a 'true' SPT is defined as an independently evolved carcinoma.[17]

ORAL FIELD CHANGES

Morphological changes.

In 1962, Nieburgs et al. reported malignancy-associated changes within smear cells of normal buccal mucosa in patients with malignant disease. The changes consisted of an increase in nuclear size, discontinuous nuclear membrane, numerous Feulgen-negative areas, increased associated chromatin surrounding the clear areas, and absence of a single large nucleolus.[18] Incze et al. confirmed the increase in nuclear area in normal oral mucosa remote from HNSCCs using ultrastructural analysis. They also described an altered nuclear to cytoplasmic area ratio.[19] A reduction in cytoplasmic area was later shown by Ogden et al. They suggested that tobacco might play a role in this alteration. However, they could only show a nonsignificant tendency for the influence of tobacco and alcohol on this morphological change in the HNSCC patients.[20]

Aneuploidy and Chromosomal Aberrations.

In the last decade, other field changes have been reported. Although polyploid cells were not detected in normal tumor-distant mucosa, aneuploidy was observed in hyperplastic/inflammatory mucosa that subsequently developed in an invasive carcinoma.[21] This aneuploidy was not detected in hyperplastic/ inflammatory mucosa from healthy individuals. Hittelman et al. determined by using chromosome in situ hybridization that genomic instability in the upper aerodigestive epithelial field increases the risk to develop a HNSCC.[22] Chromosome aneusomies were detected, aneusomies of chromosomes 2, 6, and Y was observed in the mucosa from smokers.[23] In another study, polysomies of chromosomes 7 and 17 were observed in TAM from HNSCC patients.[24]

A significant loss of chromosome Y was detected in TAM from smoking HNSCC patients, but this loss appeared not to be present in the nonsmoking patients.[25] In another publication on chromosomal aberrations in HNSCC patients, the investigators used microsatellite analysis. Allelic loss of chromosome 13 was detected in 10 of 16 informative TAM samples when they were compared to blood samples. No data on a relationship of this finding with smoking could be detected.[26]

Alterations in Cytokeratin Expression.

Aberrant expression of cytokeratins has been shown during the process of HNSCC carcinogenesis. [27] Presence of cytokeratins 7, 8, 13, 16, and 19 was observed at abnormal anatomical sites

or at abnormal intraepithelial levels in normal mucosa from HNSCC patients.[28] Only one study is available in which cytokeratin expression was studied in relation to smoking habits. Expression of cytokeratins 7 and 8 in TAM occurred more frequently in the smoking group of patients than in the nonsmoking group.[29]

Changes in Blood Group Antigens of the ABH System.

Type 2 chain ABH-carbohydrate structures are distributed broadly in epithelial and endothelial cells, independent of the patient's ABO blood group. In normal oral and laryngeal epithelium, type 2 chain ABH antigens are expressed on parabasal cells.[30] A 4-fold lower expression of type 2 chain ABH-antigen was shown in exfoliated cells from macroscopically normal mucosa from six different places distant from the HNSCC, compared with healthy individuals.[31] Because the ABH type 2 chain expression was always lower in the mucosa from the patients than in the mucosa from healthy controls, this antigen may be promising as a negative marker for field change and risk indication. [31]

Foci of Cyclin D1 Expression.

Cyclins are cell cycle regulators that are functional only when associated with CDKs. Cyclin D1 regulates the G1-S transition in the cell cycle and is functional when it is associated with either cdk4 or cdk6. [32] Amplification of the chromosome 11q13 region, which results in over expression of the proto-oncogene cyclin D1 has been described in about half of the HNSCC. Cyclin D1 amplification has been shown in premalignant lesions and the amplification frequency progresses from premalignant lesions to invasive carcinoma. [33]

Increased Expression of the Epidermal Growth Factor Receptor.

One of the cellular oncogenes that play a role in the development of HNSCC is the EGFR. This gene encodes the receptor of the growth factors epidermal growth factor and TGFa. Ligand binding to the extracellular domain of the EGFR causes receptor dimerization, which activates tyrosine kinase function. This leads to autophosphorylation and subsequent phosphorylation of intracellular target proteins, which results in proliferation.[34] EGFR mRNA overexpression, as well as protein overexpression, has been demonstrated in nearly all HNSCCs.[35]

Elevated TGFa mRNA.

Besides investigation of the EGFR also one of its ligands, TGFa , was investigated. It was shown that the mRNA level of TGFa was 5-fold increased in normal. TAM compared with mRNA levels in control normal mucosa, but whether this relates to smoking is unknown. [35]

Increased Proliferation.

One of the characteristics of a tumor is an increased proliferation. Shin et al. showed a sequential increase in proliferating cell nuclear antigen expression in head and neck tumorigenesis. [36]

p53 Overexpression.

Loss of function of the tumor suppressor p53 can result in uncontrolled cell division and progressive genomic instability. Abnormalities of the p53 tumor suppressor gene are among the most frequent molecular events in cancer. More than 90% of the HNSCCs contain mutated p53, and in 50% of the tumors, LOH of p53 has been shown. Mutant p53 has a higher stability than wild-type p53, which allows accumulation to levels detectable by immunohistochemistry. The frequency of p53-positive cells gradually increases as oral epithelium progresses from normal to hyperplasia to dysplasia to carcinoma.[37]

Lack of bcl-2 Expression.

bcl-2, an apoptosis inhibitor, and its family members (among others, bax, an apoptosis inducer) play an important role in the regulation of the apoptotic pathway. Apoptosis itself did not vary significantly in the different stages of HNSCC tumorigenesis.[38] However, there was lack of bcl-2 expression in HNSCC and in normal TAM compared to control mucosa. No data on relationship with smoking were mentioned. Because bcl-2 is supposed to inhibit apoptosis, one would expect an increase in bcl-2 expression during tumorigenesis and therefore the lack of bcl-2 expression is rather surprising. However, to estimate the bcl-2 activity, the expression of bcl-2 has to be interpreted in the context of levels of other bcl-2/bax family members. [38]

Increased Glutathione S-Transferase.

Glutathione S-transferase m is an isozyme with a marked specificity for catalyzing the conjugation of epoxides, such as benzo(a)-4,5-oxide and sterene-7–8-oxide, carcinogenic components in cigarette smoke. The expression of all glutathione S-transferase isoenzymes was significantly higher in the suprabasal and superficial layers of normal oral mucosa from HNSCC patients who subsequently developed a second primary tumor than in normal oral mucosa from HNSCC patients who were free of disease for at least 7 years. Also, in cell scrapes of macroscopically normal TAM, elevated levels of glutathione S-transferase m - and p -class were observed.[39]

Protein Tyrosine Kinase and Protein Tyrosine Phosphatase Activity.

Phosphorylation of proteins on tyrosyl residues is a key mechanism in signal transduction pathways that control growth, differentiation, and cellular architecture of normal and malignant cells.[40] This phosphorylation is strictly regulated by protein tyrosine kinases and protein tyrosine phosphatases. Normal TAM showed a 2.2-fold increase in protein tyrosine kinase activity

compared to the control mucosa from healthy individuals. In addition, in the TAM, a 1.7-fold elevated ratio of protein tyrosine kinase activity to protein tyrosine phosphatase activity was observed.[41]

CLONALITY STUDIES

As outlined in the Introduction, comparing the genetic alterations occurring in MPTs of the head and neck area will also be helpful in assessing the strength of either the migration or the independency theory in explaining oral field cancerization. If multiple tumors develop due to migration of malignant cells from a primary source, then the tumors and dysplasias from the same patient should show identical genetic alterations, whereas in case of independent origin, these alterations will be different. For these studies, various clonal markers have been used.[37]

CLONAL MARKERS.

To investigate the relationship between MPTs, good clonal markers are needed. To qualify as a marker, such a genetic alteration should (a) occur very early in the development of the primary lesion, (b) be maintained during progression of the lesion, (c) exhibit sufficient variability, and (d) be applicable in the majority of the lesions.

Some of the markers are: Xchromosome inactivation, karyotypes of tumors, LOH patterns for microsatellite markers at different chromosomal loci and p53 mutations .[37]

IMPLICATIONS FOR THERAPY

It is a well-known clinical experience that after surgical removal of a tumor, there is still a high risk for another tumor in the same anatomical area. For some cases the new tumor is explained by the growth of incompletely resected carcinoma. However, for the cases where the tumor had radically been removed it seems logical to assume that a genetically altered field is the cause of new cancer. The presence of a field with genetically altered cells appears to be a continuous risk factor for cancer. Clinical investigations are hampered by the fact that a field needs to be detected with molecular biological techniques or nonroutine visualization techniques, like fluorescence in situ hybridization.[42] Additional research is needed to identify the fields that carry the highest risk for cancer. Besides host factors, like the amount of cigarettes smoked , the biological characteristics of the field itself might be of importance for HNSCC development. Patients who have been surgically treated for HNSCC and are at risk for SFT can be enrolled to study the risk profile of a genetically altered field. A clinical trial of this type has an obvious advantage: it is known approximately where the lesion will develop (where the tumor has been), and it is possible to monitor the disease process (for instance by brushing cells). Furthermore, knowledge of the genetic alterations that precede the development to cancer will provide a basis for a rational therapy (e.g., a gene-therapy based approach) of 9these preneoplastic lesions. An

important clinical utility of field cancerization is in complementary evaluation of pathologic biopsy specimen. Currently, biopsies for cancer diagnosis are reviewed by histology, the gold standard, and the absence of abnormal cells often precludes the diagnosis of cancer. However, histologically normal biopsy specimen that possess molecular signatures of cancer fields suggest either the tumor was missed by the biopsy procedure, or that some cells in the tissue are progressing towards malignancy. Such high risk patients will require close surveillance for early detection of disease. [43]

CHEMOPREVENTION

Whether they are clonally related or not, it is clear that there are wide fields of mucosa that undergo genetic alterations in patients. It would not be feasible to remove all of the areas with molecular alterations surgically. Thus, using the knowledge gained from molecular studies, researchers have attempted to come up with protective measures that could render the mucosa less sensitive to DNA alterations. Patients at risk could be treated to prevent the development of disease, and patients with premalignant lesions could have them reversed or halted. And finally, chemoprevention could be used to prevent the recurrence of cancer after surgery. There have been several proposed compounds thought to be potential chemotherapeutic agents, but perhaps the most widely studied compound in the upper aerodigestive tract has been 13-cis retinoic acid. This family of chemicals has been shown to play a role in the differentiation, development, and growth of epithelial cells.[44] 13-cis retinoic acid has been shown to up-regulate the retinoic acid receptor-β, leading to a good clinical 26 response in head and neck pre-malignant lesions. While the focus of clinical trials for chemoprevention agents has been on the use of retinoid based compounds, the toxicity (conjunctivitis, mucositis, dry skin, hypertriglyceridemia, and malaise) of this drug at higher doses may limit its utility. Other compounds, such as cyclooxygenase-2 (COX-2) inhibitors, are being studied as chemopreventive agents because of a known increase in COX- 2 expression in patients with head and neck cancer as well as in normal epithelium adjacent to tumors.[45]

CONCLUSION:

Field cancerization is a well known and well documented process of malignant transformation. The probability that a patient with a history of head and neck squamous cell carcinoma should develop a second primary tumor in that territory is large, even after a long period from the initial tumor treatment, particularly with continued exposure to external carcinogens (smoking and alcohol abuse). Therefore, clinical screening and controlled biopsy punches are mandatory for postoperatively detected lesions in these patients; those at high risk (smokers and chronic alcohol consumers) must be present more

frequently to their dentist or primary care physician for common examinations. An important clinical implication is that the field often remains after surgery of the primary tumor and may lead to new cancers. Thus the diagnosis and treatment of epithelial cancers should be focused not only on the tumor but also on the field from which it developed.

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