Second Field Tumors: A New Opportunity for Cancer Prevention?

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Abstract
Recent molecular genetic studies provide evidence that the majority of, if not all, head and neck squamous cell carcinomas (HNSCCs) develop within a contiguous field of preneoplastic cells. Cells of a field show genetic alterations associated with the process of carcinogenesis. A subclone in a field gives rise to an invasive carcinoma. An important implication of this knowledge is that, after surgery of the initial carcinoma, part of the field may remain in the patient. A field with preneoplastic cells that share genetic alterations with cells of the excised tumor has been detected in the resection margins of at least 25% of patients, indicating that this frequently occurs. Fields can be much larger than the actual carcinoma, sometimes having a diameter >7 cm. When a field remains after resection of the tumor, the risk for another carcinoma, designated as a second field tumor (SFT), is considerably greater. It is important to realize that an SFT develops from preneoplastic cells clonally related to the initial tumor. In this respect, it should be discriminated from a recurrent carcinoma that has developed from minimal residual cancer that was left behind and from a second primary tumor that independently develops from the initial carcinoma. Patients at risk for SFTs belong to a unique patient group for whom intense surveillance is indicated and chemoprevention is an attractive option. The priorities are to identify the patients in whom a remaining field will progress to cancer and to find the genes involved. With this knowledge, highly efficient clinical prevention trials, including those using the local application of therapeutic agents, can be designed. It is important to note that SFTs also may occur after treatment of various other cancers, including those of the bladder, skin, esophagus, lung, cervix, breast, and colon. The Oncologist 2005;10;493–500

Introduction
Head and neck squamous cell carcinoma (HNSCC) develops in the mucosal lining of the oral cavity, pharynx, larynx, and cervical esophagus and comprises about 5% of all newly diagnosed cancer cases in developed countries [1]. Worldwide, there is a prevalence of approximately

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In general, one third of HNSCC patients present with early-stage (I and II) disease, whereas the remaining have advanced disease (stages III and IV) at presentation. Early-stage HNSCC can be cured with surgery or radiotherapy in a great majority of cases. Patients with advanced disease, however, are mostly treated with a combination of surgery and radiotherapy, or radiotherapy with or without chemotherapy, and have a much worse prognosis. Despite significant advances in local tumor control, the long-term survival of HNSCC patients has only moderately improved during the past two decades [3]. An important reason for this lack of progress is the relatively high tumor recurrence rates observed in these patients. Locally recurrent cancer occurs in 10%–30% of patients with advanced disease [4]. Moreover, approximately 10% of patients develop regional recurrence, while 15%–25% are confronted with distant metastases [5]. Another type of failure is the development of second primary tumors (SPTs) in the respiratory system and upper digestive tract [6]. These SPTs occur with a constant rate of 2%–3% per year [7].

Thus far, clinicians have relied on the histopathological assessment of HNSCC and its margins to make decisions regarding adjuvant treatment and prognosis. The elucidation of molecular processes that play a role in head and neck carcinogenesis may result in the development of more reliable markers of disease and more tailored treatments, thus allowing for improved survival. This review focuses on new molecular insights into the mechanisms by which recurrent or secondary cancer develops at the same or an adjacent site after curative surgery of an initial HNSCC. It is shown that the presently used clinical definitions of local recurrence and SPT have shortcomings, and therefore, new definitions are proposed that take the molecular genesis of these lesions into account.

**Current Clinical Definition of Locally Recurrent Cancer**

After surgical removal of an HNSCC, patients have a considerable risk for developing locally recurrent cancer. To differentiate between local recurrence and an SPT, local recurrence is defined, according to clinical criteria, as occurring at a distance <2 cm from the initial tumor and within 3 years after the index tumor [8]. After surgery of a primary HNSCC, the resection margins are examined by a pathologist to identify any residual cancer. If residual cancer is found, there is a relatively high risk for the development of recurring cancer, and therefore, additional therapy, re-excision, or postoperative radiotherapy is indicated [9]. Unfortunately, locally recurrent cancer develops even when resection margins are histologically tumor-free. It is believed that the relatively small number of cancer cells that remains in the patient at the margin is the main source of local recurrence. This limited number of cells has been designated local minimal residual cancer (MRC) [10]. A further concern when investigating the margins of the initial HNSCC is the presence of dysplasia, a preneoplastic condition. Dysplasia is scored according to standard criteria of the World Health Organization as mild, moderate, or severe [11]. A severely dysplastic lesion (i.e., carcinoma in situ) is often considered an indication for further treatment. The importance of the presence of moderate or mild dysplasia, however, is less clear. No further treatment is generally given, although there are reports showing that moderate and mild dysplasia can develop into cancer [12–14]. In addition, despite standardization, histological classification remains subjective and, as such, has a low predictive power [15, 16]. For the individual patient, histological grading is, therefore, considered to be of limited value.

**Current Clinical Definition of SPTs**

Besides the clinical problems related to the index tumor, HNSCC patients are at 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 [17], which were published in 1932: (a) Each of the tumors must present a definite picture of malignancy, (b) each must be distinct, and (c) the probability of one being a metastasis of the other must be excluded. 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 [8]. 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 [18]. SPTs can be divided into two groups: synchronous SPTs, which develop simultaneously with or within 6 months after the index tumor, and metachronous SPTs, which develop >6 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 [19, 20]. A recent molecular study has shed light on how this process occurs and is discussed in more detail below [21].
Genetic Progression of HNSCC

To date, it is widely accepted that an accumulation of genetic and epigenetic alterations in oncogenes and tumor suppressor genes forms the basis for the progression of a normal cell to a cancer cell, referred to as the process of multistep carcinogenesis. HNSCC cells are genetically unstable and often display extensive chromosomal changes, including amplifications, deletions, and translocations. The number of genetic alterations is known to increase as the disease progresses, when judged by histopathological examination. A review of the important genetic alterations in head and neck carcinogenesis development has recently been published [22]. Analysis of loss of heterozygosity (LOH) and comparative genomic hybridization have identified a number of chromosomal areas, for which most of the gene(s) have not been identified as yet. Chromosomal losses at 3p21, 9p21, 13q21, and 17p13 and gains at 3q26 and 11q13 are frequently detected in large panels of head and neck carcinomas [23, 24]. LOH at 17p13 is thought to involve TP53, whereas LOH at 9p21 is thought to involve INK4a, the tumor suppressor gene encoding p16. Losses at chromosomal regions 3p, 9p, and 17p are considered events that have occurred in head and neck epithelium relatively early on in the carcinogenesis process [25].

Genetic alterations associated with the process of head and neck carcinogenesis have the potential to be used for improving diagnostic ability. Currently, they can be of additional value for cancer risk assessment of precursor lesions [13, 26] and predicting prognosis [22, 23]. In addition, because each tumor has quite a unique pattern of alterations, this information can be used to assess the clonal relationship between lesions in a single patient.

Field Cancerization with a Genetic Dimension

The development of locally recurrent cancer and SPTs has frequently been explained by the concept of “field cancerization.” In their classic paper, Slaughter et al. used the term field cancerization for the first time in a study of 783 patients with oral cancer [27]. Based on histological examinations, field cancerization was described as follows: (a) oral cancer develops in multifocal areas of precancerous change, (b) histologically abnormal tissue surrounds the tumors, (c) oral cancer often consists of multiple independent lesions that sometimes coalesce, and (d) the persistence of abnormal tissue after surgery may explain SPTs and local recurrences. This publication has often been cited in the context of SPTs. The terms field cancerization and “field effect” were used when (pre)neoplastic processes at multiple sites were described, and it was often assumed that these had developed independently.

Genetic analyses have recently been performed to substantiate the observation of Slaughter et al. [27] that tumor-adjacent tissue can be aberrant. Many investigators have found cancer-associated genetic alterations in tumor-adjacent “macroscopically normal” tissue and surgical margins. Analyses of LOH, microsatellite instability, chromosomal instability, and mutations in the TP53 gene have been used to detect these alterations and identify the “field at risk” [25]. In addition, a detailed quantitative analysis has been performed on a group of unselected patients with oral and oropharyngeal cancers by measuring LOH and the mutation of the TP53 gene [28]. It was shown that at least one third of consecutive tumors had tumor-associated genetic alterations in a biopsy from the macroscopically normal mucosa adjacent to the tumor. In the majority of these cases (25% of the total number), the genetically altered cells could also be found in the margins of the specimen that was removed by the surgeon, indicating that genetically altered cells remained in the patient. Because only a limited part of the mucosa was sampled, and only a limited number of markers was analyzed, the real frequency of “fields” (lesions with genetically altered cells) may have been >25%. The fields showed allelic loss at various chromosome arms, particularly at 3p, 9p, and 17p. Similar patterns of aberrations (in particular, the type of mutation in the TP53 gene) in the field lesion and the corresponding tumor revealed a genetic relationship for almost all cases, indicating a common clonal origin of the field and the corresponding carcinoma [28].

The current molecular biological findings with regard to field cancerization complement the genetic progression model of head and neck carcinogenesis well. As a first step, a stem cell in the mucosa acquires genetic alterations and develops into a “patch,” a clonal unit consisting of the stem cell and its daughter cells that share the alteration. Next a preneoplastic field arises through the processes of clonal selection and expansion and replaces the normal epithelium [25]. Subsequent alterations occur, and eventually a subclone develops into cancer within the field of genetically altered preneoplastic cells. Figure 1 shows the model of how an HNSCC develops from a patch via a field to invasive carcinoma. In addition, the clinical implications of the model, as discussed below, are clarified.

As stated above, histological grading of epithelial dysplasia has been used to predict the risk for cancer thus far, but it has its limitations. The predictive ability may be improved by the incorporation of a molecular marker panel. In a recent study, standard histology was performed on resection margins and compared with molecular analyses, that is, LOH at 3p, 8p, 9p, 13q, 17p, and 18q, and Ki-67 staining [29]. It was reported that: (a) normal tissue
Second Field Tumors

Figure 1. Model of head and neck squamous cell carcinoma development of primary carcinoma and second field tumor. (A): A clonal unit within squamous epithelium is shown, above connective tissue. A stem cell (S) and transit amplifying cells (T) in the basal layer continuously give rise to more superficial cells. A stem cell is hit by a carcinogenic event (e.g., tobacco smoke, virus, or radiation), and its DNA becomes mutated. (B): All cells of the clonal unit governed by the stem cell have mutated DNA. (C): Top view: a stem cell is hit again, and more genetic damage occurs. (D): More hits further damage the stem cell. (E): A clonal unit with damaged DNA has crossed a border: expansion at the expense of healthy mucosa. A field with genetically altered cells develops. (F): A subclone in a field turns into a cancer cell. Uncontrolled growth starts. (G): An invasive carcinoma develops. (H): The initial carcinoma is removed by the surgeon. (I): After treatment, a field has unfortunately been left behind, and it is inadvertently unnoticed by the surgeon. (J): A cell in the field is hit and turns into a cancer cell. (K): Another carcinoma has developed in the same field: a second field tumor.

(no dysplasia) could contain genetically altered cells, (b) one third of the lesions scored as mild dysplasia did not contain genetic alterations, (c) LOH was more frequent in higher grade lesions, and (d) assessment of proliferation with Ki-67 was a better surrogate marker for LOH than histological grading. Future prospective studies should more definitively prove the predictive value of a molecular marker panel.
Field and Second Field Tumors

Fields with genetically altered cells can be large (up to 7 cm in diameter [21]) 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. This evidence has been found in retrospective analyses [21, 30], and other studies have also shown that fields have developed into tumors during follow-up [28, 31, 32]. Most available data are from retrospective analyses and is discussed here in more detail. Bedi et al. [19] and Scholes et al. [20] reported that a certain proportion of SPTs are genetically related to the first tumor and derived from a common mutated precursor cell. Various mechanisms have been proposed to explain the common clonal origin of these tumors, such as shedding of (pre)neoplastic cells into the saliva and implantation at other sites [19, 33] and lateral migration of isolated preneoplastic cells [19, 34, 35]. The most likely explanation, however, was provided when it was shown that, for six of 10 investigated tumor pairs, the first tumor and SPT (defined according to standard clinical criteria) had developed from the same preneoplastic precursor lesion (i.e., the field); in the remaining cases, the tumors had developed independently [21]. That study also showed that these fields were contiguous. The decision of whether the multiple carcinomas and the intervening field had a similar clonal origin was based on a comparison of the patterns of genetic alterations, a similar mutation in the TP53 gene, or a unique pattern of LOH. Independent tumors were completely different with respect to the genetic markers investigated. Hence, based on etiology, there are two types of SPTs: one group originating from the same field in which the first primary tumor developed and the second group having an independent origin. Because the difference in etiology has clinical implications, we propose discriminating between these two types of SPTs, with a “second field tumor” (SFT) defined as a tumor that has developed from the same field as the index tumor and a “true” SPT defined as an independently evolved carcinoma (Fig. 2).

How a seemingly locally recurrent cancer develops was investigated in an analogous way [30]. Locally recurrent cancer was defined according to current clinical criteria, having occurred < 2 cm away from the first tumor within a 3-year time period. Recurrent cancer develops from either (a) the growth of a relatively small number of tumor cells that have not been detected by the pathologist or (b) a precursor lesion that was the source of the primary tumor and in which additional genetic alterations have again led to invasive cancer. In that retrospective study of 13 HNSCC cases, the genetic pattern of the primary tumor, the surrounding histologically tumor-free resection margins, and local recurrences were analyzed. A precursor lesion was absent in five of the cases (39%), and the genetic similarity between the primary and recurrent cancers was high, providing evidence that residual cancer cells were the origin of recurrence. For the remaining eight cases (61%), a genetically related precursor lesion (field) was detected, and for five of these cases evidence was found that both the primary and recurrent carcinomas originated from this field. That study also showed that a field resulted in the development of a subsequent tumor, an SFT.

Recent molecular findings allow for a more refined classification of the lesions that can develop after an HNSCC has been surgically removed [8]. Thus, a patient can develop a true local recurrence, an SFT, or a true SPT; this classification being based on the decreasing clonal relationship between the first and subsequent lesions (Fig. 2, Table 1). The proposal for a new classification system based on molecular information and the relationship between this classification system and the original one based on clinical information only is presented in Figure 2. This new classification is desirable because it has implications for patient care (Table 1).

Importance of the SFT Concept for Prevention

The realization that many, if not all, HNSCCs are preceded by genetically defined precursor lesions opens new possibilities for early diagnosis and prevention. This would be particularly valuable if a subgroup of lesions could be defined with a very high risk for progression. In previous studies addressing the risk for progression of dysplastic leukoplakic lesions in the oral cavity, it was shown that specific patterns of allelic imbalance or ploidy are important markers to predict progression [13, 14, 26, 36]. This type of molecular analysis may also be very well suited to
accurate risk assessment of the clinically invisible fields that surround tumors. Once a more reliable risk assessment has been developed, it can be exploited to identify high-risk fields. Patients with such high-risk fields in surgical margins should theoretically be followed by lifelong surveillance at regular intervals. Moreover, this technique could indicate a more conservative approach to adjuvant radiotherapy as far as the primary site is concerned. Current adjuvant treatment modalities (surgery, chemotherapy, and/or radiotherapy) are very effective in eradicating tumor cells, but these may not be the treatments of choice for relatively large fields of preneoplastic cells [37].

The concept of an SFT offers a new opportunity for cancer chemoprevention. When a set of molecular markers has been validated, chemoprevention trials can start in patients having a high risk for an SFT. Clinical trials of this type have an important advantage: approximately where the lesion will develop is known, and the disease process can be followed by taking samples in a noninvasive way, that is, by the brushing of cells [38]. The application of genetic markers opens the way for sensitive detection of aberrant cells in small tissue samples [39].

The accessibility of the mucosa of the head and neck provides the opportunity to deliver therapeutic agents directly to the tissue at risk. Topical application, also called “mouthwash therapy,” has been shown to be feasible [40]. At this moment, some promising molecular targets are being investigated in HNSCC prevention, including the epidermal growth factor and the cyclooxygenase (COX)-1 and COX-2 pathways [22]. Another new area that holds promise is the selective killing of cells that carry defects in TP53-dependent signaling pathways by means of a genetically engineered adenovirus. Activity has been observed when ONYX-015 is applied topically in patients with clinically apparent and histologically dysplastic lesions of the oral mucosa [40].

Patients who have been treated for HNSCC are not only at risk for an SFT but also for an independent, new tumor, an SPT. Patients at risk for an SPT constitute a different patient group from the prevention point of view. Investigations of the tissue at risk are impractical because it is hard to predict precisely where a second primary will develop; this can be at more remote sites such as the lungs. For this type of patient, it may also be harder to define a risk profile than for the patient at risk for an SFT. With respect to chemoprevention, it may be that patients at risk for an SPT could profit from a more systemic approach.

The concept of SFT may also be applicable to other cancer types. In other organ systems, genetically altered cells have been detected in normal epithelium in association with an increased risk for developing multiple tumors. Fields have been described in the lung, skin, esophagus, colon, breast, and bladder [41–48]. Thus it seems that the way is open for a chemopreventive approach for the prevention of an SFT in other patient groups as well.

In conclusion, HNSCCs are often preceded by large precursor lesions. This may also be true for a significant number of other tumor types. Once a carcinoma has been removed by the physician, the presence of a remaining field with a large number of preneoplastic cells is likely to increase the risk for an SFT at that particular or an adjacent site. Detection and monitoring of these fields at risk and the development of a targeted molecular intervention may have profound implications for cancer prevention.

**Disclosure of Potential Conflicts of Interest**

The authors indicate no potential conflicts of interest.
REFERENCES


