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REVIEW ARTICLE

A genetic progression model of oral cancer: current evidence and clinical implications

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Based on recent genetic studies, we propose a progression model for the development of oral squamous cell carcinoma. In the initial phase, a stem cell acquires a genetic alteration; subsequently a patch is formed, a clonal unit consisting of the stem cell with its daughter cells that all share the DNA alteration. The next critical step is the conversion of a patch into an expanding field as a result of additional genetic alterations. This mucosal field replaces the normal epithelium and in the oral cavity such fields have been detected with dimensions of over 7 cm in diameter. Sometimes these fields are visible as leukoplakia. Ultimately, clonal selection leads to the development of carcinoma within this contiguous field of pre-neoplastic cells. An important clinical implication of this model is that fields often remain after surgery of the primary tumor and may lead to new cancers, presently designated by clinicians as second primary tumors or local recurrences.
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Genetic progression

In recent years considerable progress has been made in understanding the genetic basis of the development of oral squamous cell carcinoma (or shortly: oral cancer). It is well established that an accumulation of genetic alterations is the basis for the progression from a normal cell to a cancer cell, referred to as multi-step carcinogenesis (1). Progression is enabled by the increasingly more aberrant function of genes that positively or negatively regulate aspects of proliferation, apoptosis, genome stability, angiogenesis, invasion and metastasis (2). Gene function can be altered in different ways: tumor suppressor genes may be inactivated by mutation, deletion or methylation and oncogenes can be activated by mutation or amplification. A description of these alterations and how these are detected has previously been described (3–5). Oral cancers are characterized by a multitude of these genetic alterations and ongoing research is focusing on identifying the critical genetic events and the order in which they occur during carcinogenesis. Frequently occurring genomic alterations are supposed to contain the genes that are most important for the development of a certain type of cancer (6). Common alterations for oral cancer are inactivation of CDKN2A (p16 located at 9p21) and TP53 (located at 17p13), gain of chromosomal material at 3q26 and 11q13, and losses at 3p21, 13q21 and 14q32 (7, 8). For most of these regions the putative tumor suppressor genes or oncogenes still need to be identified. The frequency of these alterations in tumor and histopathologically defined precursor lesions has formed the basis for the description of the first genetic progression model for head and neck cancer (1). In general, loss of chromosomal material (allelic losses) at 3p, 9q and 17p was observed in a relatively high proportion of dysplastic lesions and therefore these alterations were interpreted to be early markers of carcinogenesis. Losses at 13q and 8p where more frequent in carcinomas than in dysplasias, indicating that these events are associated with the later stages of carcinogenesis. Several studies suggest, however, that early genetic changes do not necessarily correlate with altered morphology. Recent genetic findings prompted us to propose a un update of this progression model with important clinical implications.

Patches: early alterations of stem cells

The oral squamous epithelium is maintained throughout adult life by stem cells, defined as cells with the capacity to self-renew and to generate daughter cells that can differentiate to form all of the cell types that are found in the mature tissue (9). Thus far, stem cell biology research has mainly been performed in the hematological system, but knowledge about these cells in

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solid tissues is increasing. Although no definitive identification of stem cells in the oral mucosa has been made, studies in skin have provided important insight (10). Stem cells are responsible for tissue renewal, a process that continuously takes place in the oral mucosa and in addition they are involved in tissue damage repair. These cells are believed to be located in the basal layer and have a slow proliferative rate (9, 11). When a stem cell divides in a asymmetrical way (12), one daughter cell is completely identical and retains the capacity for self-renewal, while the other daughter cell goes through a number of cell divisions and gives rise to ultimate terminally differentiated populations. The cells intermediate between stem cells and terminally differentiated cells are known as transit amplifying cells and are located in the basal and the suprabasal layers of the epithelium. The stem cell together with its related family of daughter cells form a 'clonal unit' (13), of which the size has been estimated at 2 mm in diameter in normal human skin (14).

Most tumors are clonal in origin and it has been estimated that five events in humans are required to transform a normal cell into a cancer cell (15). So, only long time residents of the epithelium, most likely the stem cells, have the ability to accumulate the number of necessary genetic hits that will result in cancer formation. Thus, oral cancer is likely to originate from the stem cells in the oral mucosa (10).

Clusters (< 200 cells diameter) of cells can be observed in the oral mucosa with p53 immuno-staining and sequence analysis showed that the p53 gene is mutated in these cells (16). This 'patch' has been interpreted as a clonal unit of mutated cells. The stem cell has genetic damage in the form of a mutated p53 gene and has transferred this mutation to its daughter cells. Other investigators have also found evidence for the existence of mutated clonal units in oral epithelium (17). Clusters of p53-mutated cells have also been found in the normal human skin (18), and in sun-exposed skin these clusters were more frequent than in sun-shielded skin. For the skin, the size and distribution of p53 mutated patches could be compared with the distribution of clonal units, i.e. stem cells (identified on the basis of a high beta-1 integrin expression) with accompanying daughter cells (19).

We propose that a p53 mutated patch in the oral mucosa is a first manifestation of oral cancer. The chances that a single stem cell that has sustained a p53 mutation subsequently will acquire additional oncogenic alterations are very low. Two mechanisms, however, may contribute to the increase of the probability that cancer develops. First, the population with stem cell characteristics may increase and more cells are the source of subsequent clonal expansion (12, 20) and secondly, a specific genetic hit leads to a general 'genetic instability' (21).

Fields of genetically altered cells

Tumor-adjacent macroscopically normal epithelium has been found to contain genetic alterations and this was the reason to designate this tissue 'field at risk' (22, 23). By measuring loss of heterozygosity (allelic loss) and the mutation of the p53 gene, a quantitative analysis has been performed on a group of patients with oral and oropharyngeal cancer (24). It was shown that at least one-third (10 of 28) of consecutive tumors have tumor-associated genetic alterations in a biopsy taken from the macroscopically normal mucosa adjacent to the tumor. In the majority of these cases the genetically altered cells could also been found in the margins of the specimen that has been removed by the surgeon. As only a limited part of the mucosa was sampled, the real frequency of lesions with genetically altered cells, or in short 'field', may be higher. These field lesions are much larger than patches, being at least 4 mm in diameter and show allelic loss at various chromosome arms, particularly at 3p, 9p and 17p. A recent study showed that fields can have a diameter of over 7 cm (25). Detailed comparison of the aberrations between a field lesion and the corresponding tumor revealed a genetic relationship for almost all cases (24–28). On the basis of the common clonal origin of field and carcinoma, and the observation that field never shows invasive growth, it is plausible that field is the lesion that precedes cancer.

Clinical investigations are hampered by the fact that a field needs to be detected with molecular biological techniques or non-routine visualization techniques, like fluorescence *in situ* hybridization (22). However, there is an exception: some fields are visible as leukoplakia. Leukoplakia is defined as a 'predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion; some oral leukoplakias will transform into cancer' (29). The prevalence of leukoplakia is 0.1–0.2% in the normal population.

Importantly, a proportion of these lesions develop into carcinoma with a constant rate of 2–3% per year (30). Some leukoplakia lesions do contain cancer-associated genetic alterations and can be considered field by definition (26). This refers to losses at 3p, 9p and 17p, and also for mutations in the p53 gene (31), genetic alterations known to occur early in oral carcinogenesis (1). A proportion of oral carcinomas have at the time of diagnosis adjacent leukoplakia (32).

Although most of these fields with genetically altered cells are macroscopically not detectable, conventional histopathology can be helpful. With respect to the relation between the presence of genetic alterations and histopathological detection some general statements can be made (33): (i) all severe and moderately dysplasias do contain genetic alterations, and (ii) one-third of the mildly dysplastic lesions do not contain genetic aberrations and (iii) some normal epithelia do contain areas with genetically altered cells. In addition, genetically altered fields can be detected with immuno-staining. A good correlation was described between the presence of genetically alterations and the percentage of proliferating (KI-67 positive) cells (33). For other markers that have been detected with immuno-staining in carcinoma adjacent mucosa (34–36), such a correlation needs to be established.

The patch-field-carcinoma model

Current knowledge prompted us to propose the following progression model for oral cancer (Fig. 1). In the initial phase, a stem cell acquires one (or more) genetic alterations, one of which is likely to be a mutation in the p53 gene and forms a patch with genetically altered daughter cells. As a result of subsequent genetic alterations the stem cell escapes control and gains growth advantage, and the patch starts to expand in a lateral direction. Which critical hit is important for the cells of a patch to leave their natural containment is unknown as yet. Larger clones encompassing the size of several stem cell clusters have been observed in skin suggesting that clonal expansion in a lateral direction indeed do take place in squamous epithelium (10, 37).

The field lesion grows and takes over the normal epithelium, without becoming invasive. The process may be accelerated by the expansion of the genetically altered stem cell population through 'symmetrical' stem cell

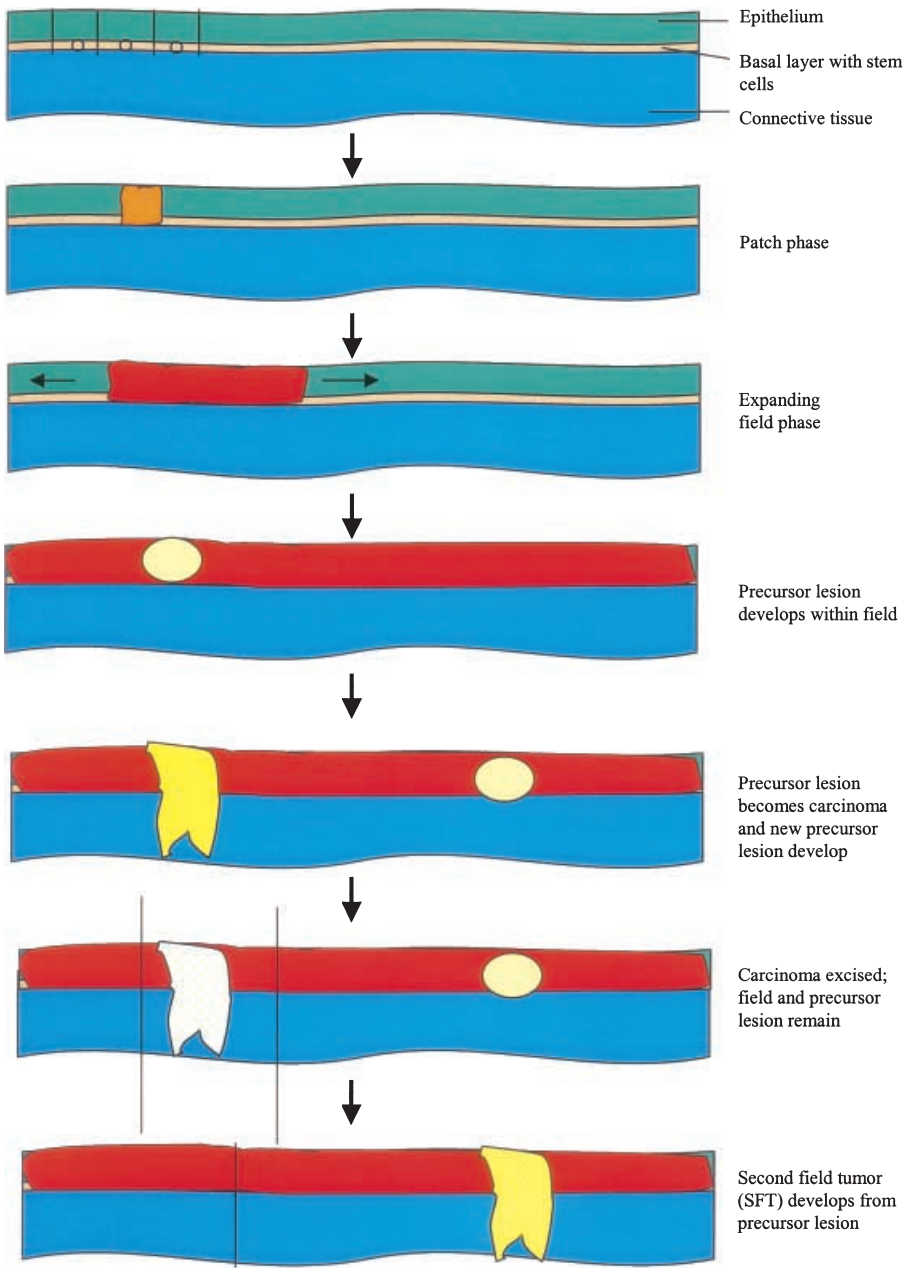

Figure 1 Schematic overview of the proposed concept of carcinogenesis of oral squamous cell carcinoma. At the top the epithelium is shown (green) with the basal layer (light orange) including the stem cells (three are shown) and connective tissue (blue). Underneath the formation of a patch (dark orange), as a clonal unit of genetically altered cells is shown. Next the formation of an expanding field (red) from a patch is visualized. Within such field of genetically altered cells a pre-neoplastic lesion develops (light yellow). In the next stage that lesion develops into cancer (yellow) and another pre-neoplastic lesion emerges. The carcinoma is resected by the surgeon, but the field and the lesion remain in the patient. At the bottom the development of a second field tumor is shown.

divisions (12). As a result more cells are target for subsequent 'hits'. Multiple clones develop within a field, and while genetic hits continue, the number of affected cells is increasing by virtue of the processes of clonal expansion and selection. Each time the daughters of the most dominant clone overtake in a wave-like fashion the rest of the cells in the field (21). So, large areas of normal mucosa are replaced by cell populations that are becoming increasingly more genetically aberrant, but are of monoclonal origin. During the process of clonal selection fields can be heterogeneous (24), because of the continuing accrual of genetic changes. These expanding fields are genetically characterized by loss of heterozygosity (LOH) at 3p12-24, 9p21 and 17p13 and a mutation in the p53 gene (24, 25), aberrations that are also observed in dysplastic leukoplakia. When the p53 gene mutation leads to over-expression of the protein the fields can be visualized by immuno-staining (16). In addition to the p53 pathway, the pRB/p16 pathway is often perturbed in fields (38). At some time point during field expansion, the process of genomic instability may enhance the speed at which mutations occur (39). The presence of a large number of genetically altered cells is a continuous threat and a certain time point the clonal section process leads to the development of a subclone with dramatic consequences: a carcinoma characterized by invasive growth and metastatic potential. Details of this ultimate transforming event have not been revealed yet, but the chance of this to happen in patient will be proportional to the number of patches and the number of additional hits. So, this proposed genetic progression model has a monoclonal origin as a strong basis (40). Two critical steps in the model can be discriminated: the conversion of a patch stem cell into an expanding population of stem cells without proper growth control and the eventual transforming event, turning a field into an overt carcinoma with invasive growth and metastasis.

Clinical implications of the patch-field-carcinoma model

The concept of carcinogenesis with an expanding field as intermediate lesion has important clinical consequences. It is a well-known experience that after surgical removal of a tumor, there is still a high risk for another cancer in the same anatomical area. For some cases this new cancer is explained by the regrowth of incompletely resected carcinoma. However, for cases where the pathologist has shown that the resection of the tumor has been radical, it is a likely possibility that the genetically altered field is the cause of new cancer. The presence of a field bears a continuous risk for cancer development. This is not merely a theoretical estimation, but in fact, it has already been shown that genetically altered fields that remain after surgery develop into cancer (24, 41, 42). This provides a new paradigm of how one should evaluate the phenomenon of another cancer after surgery of an oral carcinoma. The definitions of 'local recurrence' and second primary tumor need a genetic addendum (5). The term 'second primary tumor' was proposed to represent the second tumor that has developed independently from the first tumor. At this moment clinical criteria are used to define a second primary tumor: it developed more than 2 cm away from the index tumor or the time-interval between the carcinomas to occur was more than 3 years (5). When a second tumor arises from the same field in which a first tumor has developed, we proposed it to designate it a 'second field tumor' (SFT). It is important to make this discrimination because this different etiology may have consequences. Second field tumors will be followed relatively easily by third and fourth tumors. Therefore, a patient who has had a second field tumor may need a follow-up, characterized by frequent and more focused examinations. An analogous discussion can be considered with respect to the development of a local recurrence. These lesions are defined according to clinical criteria to occur less than 2 cm away from the primary tumor within a time period of 3 years (5). In fact, this type of lesion can be the result of remaining tumor cells but also the local remnant of field may develop into cancer (16). So, in fact a local recurrence and a second primary tumor can both have emerged from a same type of precursor lesion, a field with genetically altered cells.

It is not known what specific genetic characteristics determine the risk of a field to develop into cancer. Future research efforts should focus on identifying the genetic alterations that are responsible for this progression to invasive carcinoma. Research on low-grade oral dysplasia show encouraging results when identifying these genetic markers. It was shown that the number and type of genetic alterations is associated with an increased cancer risk (27, 28, 42). There seems to be a place for molecular grading; conventional histopathological grading has reached its plateau and needs improvement. It is generally known that histopathological grading is subjective (33) and it has limitations to predict progression to cancer (41).

Patients with a field are a potentially important target group for the study of cancer prevention. When genetic markers can be used to predict a high risk for cancer, chemoprevention trials can be started in this patient groups. Clinical trials of this type have an important advantage: it is known where approximately the lesion will develop and the disease process can be followed by taking samples in a non-invasive way, i.e. brushing of cells.

In conclusion, the presence of a field with genetically altered cells is a risk factor for oral cancer. The presence of a large number of pre-neoplastic cells is likely to increase the risk for another cancer. Detection and monitoring of a field at risk and the development of a targeted molecular intervention may have profound implications for oral cancer prevention.

References

1. Califano J, Vanderriet P, Westra W, et al. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996; **56**: 2488–92.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57–70.

3. van Houten VMM, Tabor MP, van den Brekel MWM, et al. Molecular assays for the diagnosis of minimal residual head- and-neck cancer: Methods, reliability, pitfalls, and solutions. *Clin Cancer Res* 2000; **6**: 3803–16.
4. Reid CB, Snow GB, Brakenhoff RH, Braakhuis BJ. Biologic implications of genetic changes in head and neck squamous cell carcinogenesis. *Aust N Z J Surg* 1997; **67**: 410–6.
5. Braakhuis BJM, Tabor MP, Leemans CR, van der Waal I, Snow GB, Brakenhoff RH. Second primary tumors and field cancerization in oral and oropharyngeal cancer: molecular techniques provide new insights and definitions. *Head Neck* 2002; **24**: 198–206.
6. Albertson DG, Collins C, Mccormick F, Gray JW. Chromosome aberrations in solid tumors. *Nat Genet* 2003; **34**: 369–76.
7. Gollin SM. Chromosomal alterations in squamous cell carcinomas of the head and neck: window to the biology of disease. *Head Neck* 2001; **23**: 238–53.
8. Forastiere A, Koch W, Trotti A, Sidransky D. Medical progress – head and neck cancer. *N Engl J Med* 2001; **345**: 1890–900.
9. Smalley M, Ashworth A. Stem cells and breast cancer: a field in transit. *Nature Rev Cancer* 2003; **3**: 832–44.
10. Owens DM, Watt FM. Contribution of stem cells and differentiated cells to epidermal tumours. *Nature Rev Cancer* 2003; **3**: 444–51.
11. Mackenzie IC. Retroviral transduction of murine epidermal stem cells demonstrates clonal units of epidermal structure. *J Invest Dermatol* 1997; **109**: 377–83.
12. Perez-Losada J, Balmain A. Stem-cell hierarchy in skin cancer. *Nature Rev Cancer* 2003; **3**: 434–43.
13. Garcia SB, Park HS, Novelli M, Wright NA. Field cancerization clonality, and epithelial stem cells: the spread of mutated clones in epithelial sheets. *J Pathol* 1999; **187**: 61–81.
14. Chaturvedi V, Chu S, Carrol M, Brenner JW, Nickoloff BJ. Estimation of size of clonal unit for keratinocytes of normal human skin. *Arch Pathol Lab Med* 2002; **126**: 420–4.
15. Hahn WC, Weinberg RA. Mechanisms of disease: rules for making human tumor cells. *N Engl J Med* 2002; **347**: 1593–603.
16. van Houten VMM, Tabor MP, van den Brekel MWM, et al. Mutated p53 as a molecular marker for the diagnosis of head and neck cancer. *J Pathol* 2002; **198**: 476–86.
17. Waridel F, Estreicher A, Bron L, et al. Field cancerisation and polyclonal p53 mutation in the upper aerodigestive tract. *Oncogene* 1997; **14**: 163–9.
18. Jonason AS, Kunala S, Price GJ, et al. Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc Natl Acad Sci USA* 1996; **93**: 14025–9.
19. Jensen UB, Lowell S, Watt FM. The spatial relationship between stem cells and their progeny in the basal layer of human epidermis: a new view based on whole-mount labelling and lineage analysis. *Development* 1999; **126**: 2409–18.
20. Moolgavkar SH, Luebeck EG. Multistage carcinogenesis and the incidence of human cancer. *Genes Chromosomes Cancer* 2003; **38**: 302–6.
21. Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C. The significance of unstable chromosomes in colorectal cancer. *Nature Rev Cancer* 2003; **3**: 695–701.
22. Hittelman WN, Kim HJ, Lee JS, et al. Detection of chromosome instability of tissue fields at risk: in situ hybridization. *J Cell Biochem* 1996; 57–62.
23. Hittelman WN. Genetic instability in epithelial tissues at risk for cancer. *Ann N Y Acad Sci* 2001; **952**: 1–12.
24. Tabor MP, Brakenhoff RH, van Houten VMM, et al. Persistence of genetically altered fields in head and neck cancer patients: Biological and clinical implications. *Clin Cancer Res* 2001; **7**: 1523–32.
25. Tabor MP, Brakenhoff RH, Ruijter-Schippers HJ, et al. Multiple head and neck tumors frequently originate from a single preneoplastic lesion. *Am J Pathol* 2002; **161**: 1051–60.
26. Partridge M, Pateromichelakis S, Phillips E, Emilion GG, A'Hern RP, Langdon JD. A case-control study confirms that microsatellite assay can identify patients at risk of developing oral squamous cell carcinoma within a field of cancerization. *Cancer Res* 2000; **60**: 3893–8.
27. Mao L, Lee JS, Fan YH, et al. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. *Nat Med* 1996; **2**: 682–5.
28. Partridge M, Li SR, Pateromichelakis S, et al. Detection of minimal residual cancer to investigate why oral tumors recur despite seemingly adequate treatment. *Clin Cancer Res* 2000; **6**: 2718–25.
29. van der Waal I, Schepman KP, Der Meij EH, Smeele LE. Oral leukoplakia: a clinicopathological review. *Oral Oncol* 1997; **33**: 291–301.
30. Schepman KP, van der Meij EH, Smeele LE, van der Waal I. Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. *Oral Oncol* 1998; **34**: 270–5.
31. Boyle JO, Hakim J, Koch W, et al. The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res* 1993; **53**: 4477–80.
32. Schepman K, der Meij E, Smeele L, der Waal I. Concomitant leukoplakia in patients with oral squamous cell carcinoma. *Oral Dis* 1999; **5**: 206–9.
33. Tabor MP, Braakhuis BJM, van der Wal JE, et al. Comparative molecular and histological grading of epithelial dysplasia of the oral cavity and the oropharynx. *J Pathol* 2003; **199**: 354–60.
34. Copper MP, Braakhuis BJ, de Vries N, van Dongen GA, Nauta JJ, Snow GB. A panel of biomarkers of carcinogenesis of the upper aerodigestive tract as potential intermediate endpoints in chemoprevention trials. *Cancer* 1993; **71**: 825–30.
35. Bryne M, Gravdahl C, Koppang HS, Kjaerheim A, Dabelsteen E. Is the carbohydrate sialosyl-Tn a marker for altered, non-malignant activity in squamous epithelium in the head and neck region? *J Pathol* 1995; **175**: 237–42.
36. Bongers V, Snow GB, de Vries N, et al. Second primary head and neck squamous cell carcinoma predicted by the glutathione S-transferase expression in healthy tissue in the direct vicinity of the first tumor. *Lab Invest* 1995; **73**: 503–10.
37. Zhang WG, Remenyik E, Zelterman D, Brash DE, Wikonkal NM. Escaping the stem cell compartment: sustained uvb exposure allows p53-mutant keratinocytes to colonize adjacent epidermal proliferating units without incurring additional mutations. *Proc Natl Acad Sci USA* 2001; **98**: 13948–53.
38. Kresty LA, Mallery SR, Knobloch TJ, et al. Alterations of p16(ink4a) and p14(arf) in patients with severe oral epithelial dysplasia. *Cancer Res* 2002; **62**: 5295–300.

39. Sieber OM, Heinimann K, Tomlinson IPM. Genomic instability – the engine of tumorigenesis? *Nat Rev Cancer* 2003; **3**: 701–8.
40. Braakhuis BJM, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res* 2003; **63**: 1727–30.
41. Sudbo J, Kildal W, Risberg B, Koppang HS, Danielsen HE, Reith A. DNA content as a prognostic marker in patients with oral leukoplakia. *N Engl J Med* 2001; **344**: 1270–8.
42. Rosin MP, Cheng X, Poh C, et al. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin Cancer Res* 2000; **6**: 357–62.