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Editorial Comment

Editorial comment on 'Frequent 3p allele loss and epigenetic inactivation of the *RASSF1A* tumour suppressor gene from region 3p21.3 in head and neck squamous cell carcinoma' by Hogg and colleagues

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Most head and neck squamous cell carcinomas (HNSCC) are tobacco- and alcohol-related. Although significant progress has been made, many questions about the exact pathways and genes involved in carcinogenesis remain unsolved. This is probably due to the heterogeneity of the tumours caused by the prolonged mutagenesis of tobacco. Apart from carcinogens like tobacco, in 5–30% of the oral and oropharyngeal tumours there is a relationship with HPV-16 infection [1], whereas in nasopharyngeal carcinomas, Epstein–Barr virus (EBV) seems to play a role [2]. In most sporadic cancers, head and neck cancer is caused by a gene-environment interaction. It has been shown that certain genotypes, involving carcinogen and alcohol metabolism, cell cycle control and maybe also DNA repair, predispose to the development of head and neck cancer [3,4]. Furthermore, there is a familial preponderance to develop head and neck cancer, which can be assessed using a mutagen sensitivity assay [5].

As eloquently described by Hanahan and Weinberg in Ref. [6], a cancer cell can develop via several routes. The exact mechanisms involved in head and neck carcinogenesis are not yet known [7]. In this issue of the *European Journal of Cancer,* Hogg and coworkers [8] describe a well conducted study on the possible role of the *RASSF1A* tumour suppressor gene located at 3p21.3.

To develop cancer, several growth- and death-related pathways involving apoptosis, signal transduction, cell cycle control and replicative potential have to be disturbed. Furthermore, to become an infiltrative and metastasising carcinoma, proteins affecting angiogenesis and invasiveness have to be affected. It has been shown in several studies that self-sufficiency in growth signals in head and neck cancer can be caused by epidermal growth factor receptor (EGFR) overexpression [9]. As in many other carcinomas, in a premalignant stage, cells have become insensitive to antigrowth signals and avoid senescence by loss of p16 function through promoter hypermethylation and/or loss of the allele in 60–80% of the cases [10]. Furthermore, cyclin D1 is upregulated or overexpressed in 30–40% of cases, often early in carcinogenesis as well [11]. However, loss of Rb occurs in only a small minority of HNSCC. Cell cycle control deregulation, but probably more prominently evasion from apoptosis, is influenced by mutations in the *TP53* gene, which occurs in some 50–60% of the cases, often in the early stages [12]. Although the mechanisms underlying the chromosomal genetic instability are complex and largely unknown, P53 might also play a role in maintaining genetic integrity [13,14]. Genes involved in spindle cell checkpoints may play an important role as well. An important cause of apoptosis suppression in head and neck cancer may be PIK3CA overexpression, which is involved in the AKT-protein kinase B (PKB) and RAS-MAP pathways.

Many other genetic changes have been described to occur relatively early in head and neck tumorigenesis. Among these, loss of 3p21, as well as other parts of 3p, have been described by several authors [15–17]. Loss of 3p segments has also been shown to be present in premalignant lesions or resection margins. However, unlike the loss of 17p or 9p, loss of 3p is not a strong predictor

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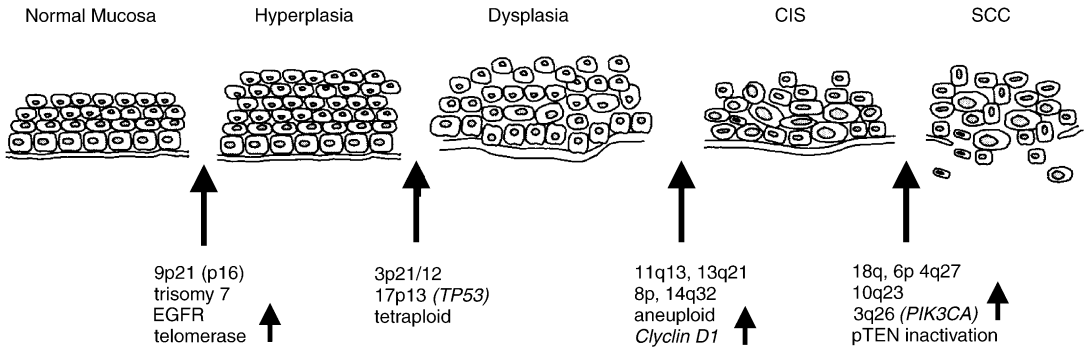

Fig. 1. Proposed model for HNSCC carcinogenesis. CIS, carcinoma *in situ*; SCC, squamous cell carcinoma.

for progression to malignant disease in these cases [18]. As the loss of a tumour suppressor gene is often caused by a mutation of the gene or methylation of the promoter and subsequent loss of the normal allele, this loss of heterozygosity (LOH) pattern indicates that almost certainly one or more tumour suppressor genes are located at 3p. Previous studies have shown that the Von Hippel Lindau (*VHL*), fragile histidine triad (*FHIT*) and *hMLH1* genes are unlikely to be the targets at 3p in HNSCC [17,19,20].

In the study of Hogg and colleagues in this issue [8], using fine deletion mapping of 3p, 81% of the HNSCC tested had allelic loss at one or more 3p loci. More specifically, 3p21.3 loss was found in 66%, whereas 3p12 loss was found in 56%. The promoter of *RASSF1A*, one of the genes located at 3p21.3, proved to be methylated in only 17%, especially in advanced, poorly differentiated tumours, whereas mutations of *RASSF1A* were not found. The authors previously reported a 34% incidence of *RASSF1A* methylation in non-small cell lung carcinomas. The *RASSF1A* gene might play an important role in oncogenesis as it is a regulator in the Ras pathway. As loss of 3p21.3 was always accompanied by loss of other 3p segments and, in half of the cases, loss of the whole allele, the authors postulate that large deletions occur as a second hit to the loss of another (not yet identified) tumour suppressor gene at 3p. In fact, *RASSF1A* is probably not the most important gene located at 3p in HNSCC carcinogenesis. However, as the authors state, a single copy of *RASSF1A* might not be sufficient to inhibit tumour development (haploinsufficiency) in case of the loss of another tumour suppressor (at 3p). The significance of subtle expression level changes is much more difficult to assess.

Although many studies have tried to elucidate the molecular carcinogenesis in head and neck cancer, many questions still remain. The studies using immunohistochemistry, *in-situ* hybridisation, microsatellite analysis, promotor methylation assays, comparative genomic hybridisation and mutation analyses of HNSCC specimens and premalignant lesions have so far come up with an incomplete model (Fig. 1). Future studies, probably using gene expression profile changes in biopsies from premalignant and malignant lesions, might reveal clues on many of the remaining questions and might be valuable in assessing the relative importance of changes in gene expression for tumour progression [21].

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