The Journal of Laryngology & Otology (2009), ***123***, *266–272*.

doi:10.1017/S0022215108003320

Review Article

Genetic profile of head and neck squamous cell carcinoma: clinical implications

F O Agada, H Patmore, O Alhamarneh, N D Stafford, J Greenman

Abstract

The outcome for patients with head and neck squamous cell carcinoma remains poor, despite improvements in diagnosis and treatment over the past three decades. This has triggered great interest in the genetic events that underpin the aetiology and clinical behaviour of this group of cancers. As a result, the genetic profile for head and neck squamous cell carcinomas at different sub-sites has been relatively well characterised at the chromosomal level. Various studies have shown links between specific aberrations in head and neck squamous cell carcinoma and clinical outcome, e.g. loss of heterozygosity at 2q and 18q is commonly associated with poor prognosis, and loss of heterozygosity at 9p21 is associated with recurrence. However, there is as yet no significant clinical application of this genetic knowledge as regards the screening, diagnosis or treatment of head and neck squamous cell carcinoma. Here, we summarise the current state of knowledge, and highlight the most promising areas of research that may facilitate the translation of genetic data into clinical benefit.

Key words: Head and Neck Neoplasms; Squamous Carcinoma; Genetics

Introduction

Head and neck squamous cell carcinoma (SCC) is the sixth commonest cancer, with a relatively high rate of local regional recurrence. Despite improvements in the diagnosis and treatment of this malignancy, the survival rate has not improved significantly over the last 30 years.[1] Many prognostic indicators have been and are currently being investigated in an attempt to define outcome at the time of diagnosis; however, as is the case for most cancers, sufficiently specific markers are hard to find.

The occurrence of nodal metastasis at presentation remains the single most important prognostic factor, being significantly correlated with poor survival rates.[2–4] Considerable increases in our knowledge of genetics over the past decade have raised hopes that deoxyribonucleic acid (DNA) based technologies may soon play a role in the early diagnosis and management of head and neck SCC. For instance, genetic testing can be performed to complement histopathological analysis at the time of initial biopsy. Rosin *et al.* have shown that such genetic testing can be used to predict the progression of oral pre-malignant lesions to invasive cancer; they have demonstrated significant differences in loss of heterozygosity patterns involving multiple genes, comparing progressive and non-progressive cases.[5]

Various studies have attempted to characterise the genetic profiles of head and neck SCC, using a variety of techniques, most commonly detection of loss of heterozygosity.[6–11] Major events in the pathogenesis of head and neck SCC have been associated with various aberrations at the genetic level which underpin key cellular activities. Despite this knowledge, the controlling mechanisms of head and neck SCC carcinogenesis and progression are still not fully understood. Califano *et al.* proposed a genetic progression model for head and neck cancer, after using microsatellite analysis which revealed that a number of genetic aberrations correlated with the various histopathological steps (i.e. benign hyperplasia to dysplasia, dysplasia to carcinoma in situ, and finally invasive cancer).[12] An updated version of this model, based on numerous study findings, is given in Figure 1.

This seminal work by Califano and colleagues has been corroborated by many other studies over the past decade, using both microsatellite analysis and, more recently, array-based comparative genomic hybridisation, in an attempt to map systematically the gene pathway for head and neck SCC.[13–15] Array-based comparative genomic hybridisation is a more recent approach used for genome-wide determination of DNA copy number alterations. Its resolution level is higher than conventional

From the Division of Cancer, Department of Otolaryngology, Head and Neck Surgery, Postgraduate Medical Institute, University of Hull, UK.
Accepted for publication: 14 June 2008. First published online 11 August 2008.

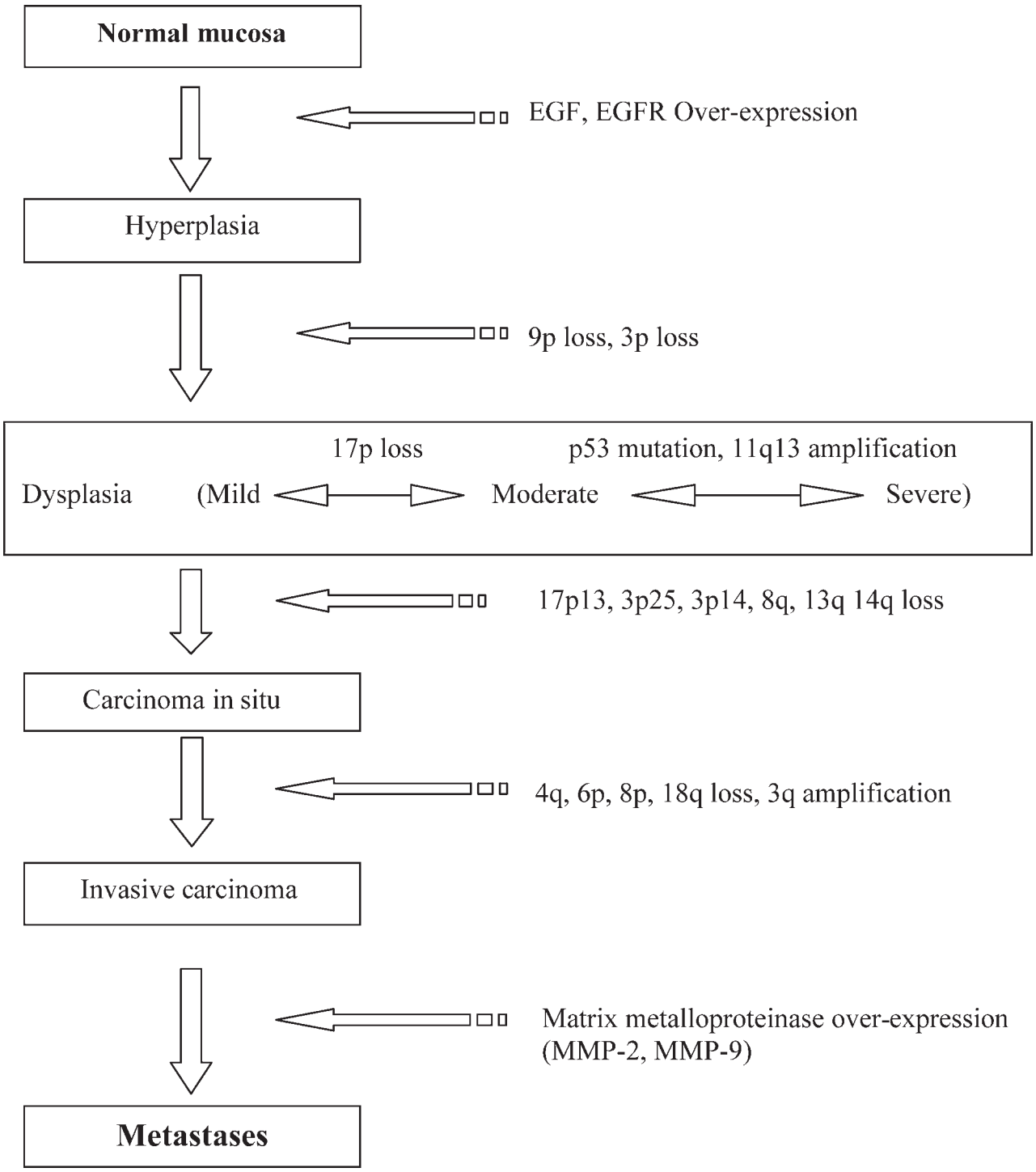

FIG. 1

Progression of normal mucosa to metastatic head and neck squamous cell carcinoma (HNSCC), with associated genetic changes. Based on the work by Califano *et al.*[12] and El-Nagger *et al.*[52], recently updated by Stafford *et al.*[53] EGF = epidermal growth factor; EGFR = EGF receptor; MMP = matrix metalloproteinase

chromosome-based comparative genomic hybridisation (CGH) methodology, 1–2 Mb *vs* 10–20 Mb level respectively.[15]

One of the major difficulties in head and neck SCC research is the fact that the various sub-sites behave differently at both the biological and the clinical level. An interesting study by Huang *et al.* has shown that different chromosomal aberrations apparently play significant roles in the initiation and/or progression of SCC at different sites.[16] This study, using comparative genomic hybridisation, studied 75 head and neck SCC patients (18 pharyngeal SCCs, 23 laryngeal SCCs and 34 oral SCCs). They concluded that 'the most important chromosomal events for progression of head and neck cancer were: +3q, +5p, +8q and −3p for all subgroups

https://doi.org/10.1017/S0022215108003320 Published online by Cambridge University Press

TABLE I

STUDIES ANALYSING CHROMOSOMAL LOSS OF HETEROZYGOSITY AND ASSOCIATION WITH PROGNOSIS: 1994–2008

Chromosomal loci	Study	Comments
3p & 9p21	Chang & Califano[54]	Elevated risk of pre-malignant lesion becoming malignant
11p & 3p	Choi *et al.*[55]	Associated with overall survival
18q	Takebayashi *et al.*[56]	Aggressive tumour behaviour
8p11–21	Coon *et al.*[57]	Decreased overall survival
3p	Yamamoto *et al.*[58]	Poor prognosis when any LOH is found at 8p, 13q or 17p
8p23	Bockmühl *et al.*[59]	Decreased overall survival
4q	Shah *et al.*[60]	Involved in disease progression
9p21	Matsuura *et al.*[61]	Associated with recurrence (CDKN2)
9p21	Lydiatt *et al.*[62]	Associated with recurrence
2q	Ransom *et al.*[63]	Poor prognosis
18q	Pearlstein *et al.*[64]	Poor prognosis
14q	Lee *et al.*[65]	Poor prognosis
8p23	Scholnick *et al.*[66]	Decreased overall & disease-free survival
3p 8p, 13q & 17p	Li *et al.*[67]	Poor prognosis when LOH found at >2 loci

Data obtained by a comprehensive search of PubMed, Ovid and Google, using the key words 'genetic profile', 'loss of heterozygosity', 'prognosis', 'head and neck', 'oral', 'hypo-pharyngeal', 'laryngeal', 'squamous cell carcinoma' and 'head and neck squamous cell carcinoma'. LOH = loss of heterozygosity

of [head and neck] SCC; additionally, +7q, +17q, −9p and −13q were important for [pharyngeal] SCC; +7p, +9q, +11q12–13, +14q and +17q for [laryngeal] SCC; and +1p and +11q12–13 for oral SCC', where '+' means gain and '−' means loss of the chromosome arm or sub-region. Others have undertaken similar analyses. The aim of this paper is to discuss briefly the potential for applying our current knowledge of head and neck SCC genetic profiles to clinical practice, either through DNA-based analysis or the translation of the genetic data into knowledge about potentially detectable proteins.

Current and future clinical applications

Diagnosis

The current methods for diagnosing head and neck SCC have major limitations. Usually, diagnosis involves a combination of clinical history-taking, endoscopy and, possibly, tissue biopsy. Despite arriving at a diagnosis, at this stage it is impossible to predict accurately an individual patient's outcome, as head and neck SCC tumours vary significantly in their growth behaviour and response to treatment (be it radio- or chemotherapy). It is anticipated that identification of a tumour's genetic profile, and comprehension of the implications of this, will both aid diagnosis and inform prognosis and treatment. The ability to make an early, personalised diagnosis without undergoing a significantly invasive procedure would be of great advantage to both patient and clinician.

Patmore *et al.* have shown that the most common aberrations in head and neck SCC are 3q (90 per cent), 8q (65 per cent), 1q (50 per cent), 5p (43 per cent), 2q (41 per cent) and 11q (41 per cent), and that the most common deletions are 3p (57 per cent), 1p (54 per cent), 4p (48 per cent), 13q (48 per cent), 11q (41 per cent) and 10q (37 per cent).[17] These authors assessed DNA from 23 paired specimens of primary tumour from various sub-sites and their matched lymph node metastases. In a similar study, Sparano *et al.* used array-based comparative genomic hybridisation to map the genetic profile of oral SCC.[18] They reported results as follows:

> [The] genomic regions most frequently amplified (>35 per cent) were located on 3q, 5p, 8q, 9q and 20q, although [the] regions most frequently deleted (>40 per cent) involved chromosomes 3p, 8p, 13q and 18q. Cancer-related genes altered in greater than 25 per cent of [oral] SCC samples were identified (22 amplified, 17 deleted). . . .

Other studies have shown similar findings of distinct chromosomal aberrations being associated with different types of head and neck SCC, based on the site of origin.[19,20]

Prognosis (Table 1)

The prognostic markers currently used to predict the survival and likely disease course of head and neck SCC are based on the histological tumour–node–metastasis (TNM) staging system (i.e. T stage, presence and extent of nodal metastasis, tumour site, tumour volume and thickness).[21] The drawback of using T stage and tumour site is that these are average parameters which alone are not significant predictors of survival.[22,23] Attempts at using histological characteristics (e.g. lymphovascular and perineural invasion as well as regional extracapsular spread), identified in the tumours and/or nodes as determinants of prognosis, have been helpful; however, these are also relatively non-specific and have not been shown to be independent predictors of survival.[24,25] Various studies have shown that nodal status is a bad prognostic marker and that spread to the nodes is associated with a significantly reduced time to recurrence and death.[26–28] However, all the conventional parameters are general markers which lack the ability to predict individual prognosis or response.

Patmore *et al.* have shown that dysplasia correlates with loss of heterozygosity at 3p21, 5q21, 9p21 and 17p13 in early laryngeal carcinogenesis.[17] They concluded that genomic changes in pre-malignant

https://doi.org/10.1017/S0022215108003320 Published online by Cambridge University Press

laryngeal lesions could be of potential use as markers of progression to invasive carcinoma. Others have investigated these associations and shown similar changes associated with dysplastic tissue. A comprehensive study by Bockmühl *et al.*, using comparative genomic hybridisation analysis on 113 primary head and neck SCC patients, demonstrated that gains at 3q21–29 and 11q13 and loss at 8p21–22 acted as independent prognostic markers which carried a higher statistical significance than nodal status.[29] Genetic aberrations were independent markers, and this allowed for molecular dissection of patients at low clinical risk (i.e. pN_0 and pT_2 tumours). Therefore, a sub-group of patients of N_0 status, who would not normally be treated, potentially could now be selected for aggressive adjuvant chemotherapy and treated differently due to their specific genetic phenotype. Similarly, head and neck SCC studies have consistently associated chromosome 11q13 gains with a poor prognosis.[30,31] A number of groups have attempted to identify a metastatic phenotype.[17,32] Although there is no clear consensus, largely because the study cohorts have been relatively small, a number of aberrations have been reported, such as gains at 10p11–12 and 11p and deletions at chromosomes 4q22–31, 9p13–24 and 14q in the nodal metastasis, when compared with the corresponding primary tumours.[32] These aberrations were seen in two matched tumour–node pairs assessed using a modified comparative genomic hybridisation assay, whereby DNA from the tumour and nodes was differentially labelled and then hybridised to a normal karyotype.

It is hoped in the future that genetic stratification of disease can be performed, in order to allow clinicians to determine the most appropriate treatment for patients, particularly those with early stage disease. In the same way, this information could inform decisions for patients with advanced stage disease, helping to predict whether patients will benefit from resection and reconstructive surgery or from a more conservative approach. If this is to become common clinical practice in head and neck cancer management, appropriately powered, large scale studies need to be undertaken.

Treatment

The current treatment modalities for head and neck SCC largely comprise surgery, radiotherapy and, to a lesser extent, chemotherapy. However, a number of studies (albeit early-stage) have investigated the correlation of genetic profile with response to certain treatments.

Radiotherapy and genetic profile screening for head and neck squamous cell carcinoma. Radiotherapy is a key treatment modality for head and neck SCC, both in the early and advanced stages. Up to 27 per cent of patients with T_2 tumours of the larynx are said to demonstrate locally persistent or recurrent disease at the original site, requiring salvage surgery to achieve a definitive cure.[33] It would therefore be extremely beneficial to patients if clinicians could identify, prior to treatment, whether their tumour was radiotherapy-responsive or -resistant. This would prevent a worthless therapy being given, saving the patient the associated loss in quality of life as well as saving the resources of the health service.

A study by Singh *et al.*, at the Memorial Sloan–Kettering Cancer Center, screened for genetic aberrations associated with radiation response, using comparative genomic hybridisation on five head and neck SCC cell lines after exposing the cells to a single course of radiation (400 cGy).[34] They demonstrated that no recurrent aberrations were unique to the radiation-resistant cell lines. However, the three radiation-sensitive cell lines did have recurrent gains at 7p and 17q and losses at 5q, 7q and 18q. This means that comparative genomic hybridisation analysis may enable prediction of radiation response even before treatment. However, the small number of cell lines analysed means that this result can only be considered a preliminary finding. To address the same question from a clinical perspective, Nix *et al.* assessed pre-treatment tissue biopsies from 124 patients with early stage (T_1–T_2, N_0) laryngeal SCC.[35] Patients were split into two equal-sized groups ($n = 62$); one group had failed radiotherapy (and hence were considered radio-resistant) and the other had been successfully treated. Both groups were matched for T stage, laryngeal sub-site and smoking history. Using immunohistochemistry, Nix and colleagues demonstrated that the expression of the apoptotic proteins bcl-2, bcl-XL, bax, bak and survivin was associated with radio-resistance in laryngeal cancer. The radio-resistant group overexpressed bcl-2 and bcl-XL and had a loss of bax expression in pre-treatment biopsies. Nix *et al.* reported that bcl-2 had an accuracy of 71 per cent in predicting radiotherapy outcome. Predicting radio-resistance or -responsiveness should significantly enhance outcome, as the clinician would be able to recommend conservative laryngeal surgery as an alternative first-line treatment to radiotherapy, or consider other modalities from the outset. This study confirmed the earlier work of the same group using a smaller cohort of laryngeal tumours.[36]

p53 and p53 vaccines. The p53 gene is the most commonly mutated gene in all cancers, including head and neck SCC. This subsequently leads to overexpression of a mutant form of the p53 protein. The normal p53 protein is activated in response to cellular stress, in order to arrest the cell cycle so that there is an opportunity for the damaged DNA to be repaired or, if this cannot happen, to initiate apoptosis. Therefore, when there is a mutation of the p53 gene, as in cancer, there is loss of the function of p53 protein. Numerous studies have investigated the importance of various p53 mutations in the development of head and neck SCC, because of this cancer's high prevalance.[37,38] Furthermore, it has been shown that the introduction of a wild-type p53 gene results in a promising anti-tumour strategy.[39–41]

There is evidence that human leukocyte antigen (HLA) A2 restricted cytotoxic T lymphocytes

https://doi.org/10.1017/S0022215108003320 Published online by Cambridge University Press

specific for human wild-type sequence p53 epitopes lyse tumour cells expressing mutant p53.[42–44] Therefore, treatment modalities that would target tumours over-expressing mutant p53 are being studied. Hoffmann *et al.*, using cytotoxic T lymphocytes from circulating precursor T cells from 30 HLA-A2.1 positive head and neck SCC patients and 31 controls, have demonstrated the potential of using HLA-A2 restricted cytotoxic T lymphocytes specific for human wild-type p53 epitopes which lyse tumour cells expressing mutant forms.[45] However, Hoffman and colleagues warn that '. . .*in vivo* p53-specific cytotoxic T lymphocytes might play a role in the elimination of tumour cells expressing the p53 264–272 epitope ('immunoselection'), leading to the outgrowth of 'epitope loss' tumour cells'. They also suggest that more immunogenic variant peptides of the 264–272 epitope could be used in those patients whose cytotoxic T lymphocytes do not respond against the original wild-type form. Other studies have demonstrated similar outcomes with different combinations of HLA molecules and peptides.[46,47] This remains a promising modality of treatment, although large scale clinical trials involving multiple peptides are required.

Intra-operative surgical margin analysis. One of the difficulties in surgical management of head and neck SCC is determination of whether a surgical margin is free of tumour or not. Between 10 and 30 per cent of all surgically treated patients develop local recurrence despite having a histologically free margin at the time of surgery.[48] Hence, predicting sub-clinical tumourigenesis could play a role intra-operatively in determining surgical margins and reducing local recurrence.[49] Chromosome imbalance associated with head and neck SCC malignancy can be detected between tumour margins and clinically normal adjacent cells. Barrera *et al.* have shown that this is possible using the fluorescence in situ hybridisation technique and DNA probes specific for 14 human chromosomes (1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 15, 17, X and Y).[50] However, it is important to note that this was a small study involving only 10 patients. In a similar study involving 52 patients undergoing primary resection for head and neck SCC, Nathan *et al.* assessed the proto-oncogene eIF4E (4E), which they report as being 'elevated in 100 per cent of head and neck squamous cell carcinoma tumours and [. . .] of prognostic value in predicting recurrence'.[51] They histologically examined all tissue from tumours classified as 'tumour-free' for the presence or absence of 4E, p53 and MMP-9. They found that 4E over-expression in the margins was a more sensitive predictor of recurrence compared with p53. In the future, surgical treatment of head and neck SCC could incorporate an intra-operative genetic test to ascertain tumour-free margins, based on over- or under-expression of specific genes such as 4E. Such a test would hopefully contribute to reducing the significant problem of local and regional recurrence and the associated poor outcome.

Future and conclusion

Head and neck squamous cell carcinoma at distinct sub-sites behaves very differently. Current methods of evaluation and treatment make it extremely difficult to individualise treatment. However, with our growing understanding of the genetic changes that underlie head and neck SCC development, it would seem a realistic aspiration that tumours could be characterised based on a specific genetic profile. Once this is possible, patients could receive individualised prognostic advice, with the next logical step being highly personalised treatment strategies. It is hard to predict when genetics-based diagnosis and treatment will become widespread; however, the rapid pace of advances in the field over the past decade leads the authors to believe that this will become a reality. Screening head and neck SCC prior to radiotherapy could be a common practice within the decade; however, genetics-based targeting therapies still require the development of safe and effective delivery vehicles.

References

1 Chin D, Boyle GM, Porceddu S, Theile DR, Parsons PG, Coman WB. Head and neck cancer: past, present and future. *Expert Rev Anticancer Ther* 2006;**6**:1111–18
2 Layland MK, Sessions DG, Lenox J. The influence of lymph node metastasis in the treatment of squamous cell carcinoma of the oral cavity, oropharynx, larynx, and hypopharynx: N0 versus N+ . *Laryngoscope* 2005;**115**:629–39
3 Kowalski LP. Lymph node metastasis as a prognostic factor in laryngeal cancer. *Rev Paul Med* 1993;**111**:42–5
4 Cerezo L, Millan I, Torre A, Aragon G, Otero J. Prognostic factors for survival and tumor control in cervical lymph node metastases from head and neck cancer. A multivariate study of 492 cases. *Cancer* 1992;**69**:1224–34
5 Rosin MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J *et al*. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin Cancer Res* 2000;**6**:357–62
6 El-Naggar AK, Hurr K, Huff V, Clayman GL, Luna MA, Batsakis JG. Microsatellite instability in preinvasive and invasive head and neck squamous carcinoma. *Am J Pathol* 1996;**148**:2067–72
7 Singh B, Stoffel A, Gogineni S, Poluri A, Pfister DG, Shaha AR *et al*. Amplification of the 3q26.3 locus is associated with progression to invasive cancer and is a negative prognostic factor in head and neck squamous cell carcinomas. *Am J Pathol* 2002;**161**:365–71
8 Feenstra M, Veltkamp M, van Kuik J, Wiertsema S, Slootweg P, van den Tweel J *et al*. HLA class I expression and chromosomal deletions at 6p and 15q in head and neck squamous cell carcinomas. *Tissue Antigens* 1999;**54**:235–45
9 Lee DJ, Koch WM, Yoo G, Lango M, Reed A, Califano J *et al*. Impact of chromosome 14q loss on survival in primary head and neck squamous cell carcinoma. *Clin Cancer Res* 1997;**3**:501–5
10 Takebayashi S, Hickson A, Ogawa T, Jung KY, Mineta H, Ueda Y *et al*. Loss of chromosome arm 18q with tumor progression in head and neck squamous cancer. *Genes Chromosomes Cancer* 2004;**41**:145–54
11 Califano J, Westra WH, Meininger G, Corio R, Koch WM, Sidransky D. Genetic progression and clonal relationship of recurrent premalignant head and neck lesions. *Clin Cancer Res* 2000;**6**:347–52
12 Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S *et al*. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996;**56**:2488–92
13 Pershouse MA, El-Naggar AK, Hurr K, Lin H, Yung WK, Steck PA. Deletion mapping of chromosome 4 in head and neck squamous cell carcinoma. *Oncogene* 1997;**14**:369–73

14 Roh HJ, Shin DM, Lee JS, Ro JY, Tainsky MA, Hong WK *et al*. Visualization of the timing of gene amplification during multistep head and neck tumorigenesis. *Cancer Res* 2000;**60**:6496–502
15 el-Naggar AK, Hurr K, Luna MA, Goepfert H, Hong WK, Batsakis JG. Intratumoral genetic heterogeneity in primary head and neck squamous carcinoma using microsatellite markers. *Diagn Mol Pathol* 1997;**6**:305–8
16 Huang Q, Yu GP, McCormick SA, Mo J, Datta B, Mahimkar M *et al*. Genetic differences detected by comparative genomic hybridization in head and neck squamous cell carcinomas from different tumor sites: construction of oncogenetic trees for tumor progression. *Genes Chromosomes Cancer* 2002;**34**:224–33
17 Patmore HS, Ashman JNE, Cawkwell L, MacDonald A, Stafford ND, Greenman J. Can a genetic signature for metastatic head and neck squamous cell carcinoma be characterised by comparative genomic hybridisation? *Br J Cancer* 2004;**90**:1976–8
18 Sparano A, Quesnelle KM, Kumar MS, Wang Y, Sylvester AJ, Feldman M *et al*. Genome-wide profiling of oral squamous cell carcinoma by array-based comparative genomic hybridization. *Laryngoscope* 2006;**116**:735–41
19 Noutomi Y, Oga A, Uchida K, Okafuji M, Ita M, Kawauchi S *et al*. Comparative genomic hybridization reveals genetic progression of oral squamous cell carcinoma from dysplasia via two different tumourigenic pathways. *J Pathol* 2006;**210**: 67–74
20 Oga A, Kong G, Tae K, Lee Y, Sasaki K. Comparative genomic hybridization analysis reveals 3q gain resulting in genetic alteration in 3q in advanced oral squamous cell carcinoma. *Cancer Genet Cytogenet* 2001;**127**:24–9
21 Le Tourneau C, Velten M, Jung GM, Bronner G, Flesch H, Borel C. Prognostic indicators for survival in head and neck squamous cell carcinomas: analysis of a series of 621 cases. *Head Neck* 2005;**27**:801–8
22 Baatenburg de Jong RJ, Hermans J, Molenaar J, Briaire JJ, le Cessie S. Prediction of survival in patients with head and neck cancer. *Head Neck* 2001;**23**:718–24
23 Verschuur HP, Irish JC, O'Sullivan B, Goh C, Gullane PJ, Pintilie M. A matched control study of treatment outcome in young patients with squamous cell carcinoma of the head and neck. *Laryngoscope* 1999;**109**:249–58
24 Soo KC, Carter RL, O'Brien CJ, Barr L, Bliss JM, Shaw HJ. Prognostic implications of perineural spread in squamous carcinomas of the head and neck. *Laryngoscope* 1986;**96**:1145–8
25 Unal OF, Ayhan A, Hosal AS. Prognostic value of p53 expression and histopathological parameters in squamous cell carcinoma of oral tongue. *J Laryngol Otol* 1999;**113**: 446–50
26 Zatterstrom UK, Wennerberg J, Ewers SB, Willen R, Attewell R. Prognostic factors in head and neck cancer: histologic grading, DNA ploidy, and nodal status. *Head Neck* 1991;**13**:477–87
27 Janot F, Klijanienko J, Russo A, Mamet JP, de Braud F, El-Naggar AK *et al*. Prognostic value of clinicopathological parameters in head and neck squamous cell carcinoma: a prospective analysis. *Br J Cancer* 1996;**73**:531–8
28 Shingaki S, Suzuki I, Kobayashi T, Nakajima T. Predicting factors for distant metastases in head and neck carcinomas: an analysis of 103 patients with locoregional control. *J Oral Maxillofac Surg* 1996;**54**:853–7
29 Bockmühl U, Schlüns K, Küchler I, Petersen S, Petersen I. Genetic imbalances with impact on survival in head and neck cancer patients. *Am J Pathol* 2000;**157**:369–75
30 Akervall JA, Jin Y, Wennerberg JP, Zätterström UK, Kjellén E, Mertens F *et al*. Chromosomal abnormalities involving 11q13 are associated with poor prognosis in patients with squamous cell carcinoma of the head and neck. *Cancer* 1995;**76**:853–9
31 Meredith SD, Levine PA, Burns JA, Gaffey MJ, Weiss LM, Erickson NL *et al*. Chromosome 11q13 amplification in head and neck squamous cell carcinoma. Association with poor prognosis. *Arch Otolaryngol Head Neck Surg* 1995;**121**:790–4
32 Wreesmann VB, Wang D, Goberdhan A, Prasad M, Ngai I, Schnaser EA *et al*. Genetic abnormalities associated with nodal metastasis in head and neck cancer. *Head Neck* 2004;**26**:10–5
33 Stevens G, Castle G, O'Brien CJ. Treatment of early carcinoma of the vocal cords by radiotherapy. *Australas Radiol* 1994;**38**:119–22
34 Singh B, Kim SH, Carew JF, Yu I, Shaha AR, Wolden S *et al*. Genome-wide screening for radiation response factors in head and neck cancer. *Laryngoscope* 2000;**110**: 1251–6
35 Nix P, Cawkwell L, Patmore H, Greenman J, Stafford N. Bcl-2 expression predicts radiotherapy failure in laryngeal cancer. *Br J Cancer* 2005;**92**:2185–9
36 Condon LT, Ashman JN, Ell SR, Stafford ND, Greenman J, Cawkwell L. Overexpression of Bcl-2 in squamous cell carcinoma of the larynx: a marker of radioresistance. *Int J Cancer* 2002;**100**:472–5
37 Delfino V, Casartelli G, Garzoglio B, Mereu P, Bonatti S, Margarino G *et al*. Micronuclei and p53 accumulation in preneoplastic and malignant lesions of the head and neck. *Mutagenesis* 2002;**17**:73–7
38 Shin DM, Charuruks N, Lippman SM, Lee JJ, Ro JY, Hong WK *et al*. p53 protein accumulation and genomic instability in head and neck multistep tumorigenesis. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:603–9
39 Brown L, Benchimol S. The involvement of MAPK signaling pathways in determining the cellular response to p53 activation: cell cycle arrest or apoptosis. *J Biol Chem* 2006;**281**:3832–40
40 Nikitina EY, Clark JI, Van Beynen J, Chada S, Virmani AK, Carbone DP *et al*. Dendritic cells transduced with full-length wild-type p53 generate antitumor cytotoxic T lymphocytes from peripheral blood of cancer patients. *Clin Cancer Res* 2001;**7**:127–35
41 Hoffmann TK, Donnenberg AD, Finkelstein SD, Donnenberg VS, Friebe-Hoffmann U, Myers EN *et al*. Frequencies of tetramer+ T cells specific for the wild-type sequence p53 (264–272) peptide in the circulation of patients with head and neck cancer. *Cancer Res* 2002;**62**:3521–9
42 Umano Y, Tsunoda T, Tanaka H, Matsuda K, Yamaue H, Tanimura H. Generation of cytotoxic T cell responses to an HLA-A24 restricted epitope peptide derived from wild-type p53. *Br J Cancer* 2001;**84**:1052–7
43 Chikamatsu K, Nakano K, Storkus WJ, Appella E, Lotze MT, Whiteside TL *et al*. Generation of anti-p53 cytotoxic T lymphocytes from human peripheral blood using autologous dendritic cells. *Clin Cancer Res* 1999;**5**:1281–8
44 McArdle SE, Rees RC, Mulcahy KA, Saba J, McIntyre CA, Murray AK. Induction of human cytotoxic T lymphocytes that preferentially recognise tumour cells bearing a conformational p53 mutant. *Cancer Immunol Immunother* 2000;**49**:417–25
45 Hoffmann TK, Bier H, Donnenberg AD, Whiteside TL, De Leo AB. p53 as an immunotherapeutic target in head and neck cancer. *Adv Otorhinolaryngol* 2005;**62**:151–60
46 Eura M, Chikamatsu K, Katsura F, Obata A, Sobao Y, Takiguchi M *et al*. A wild-type sequence p53 peptide presented by HLA-A24 induces cytotoxic T lymphocytes that recognize squamous cell carcinomas of the head and neck. *Clin Cancer Res* 2000;**6**:979–86
47 Asai T, Storkus WJ, Mueller-Berghaus J, Knapp W, DeLeo AB, Chikamatsu K *et al*. In vitro generated cytolytic T lymphocytes reactive against head and neck cancer recognize multiple epitopes presented by HLA-A2, including peptides derived from the p53 and MDM-2 proteins. *Cancer Immun* 2002;**2**:3
48 van Houten VM, Leemans CR, Kummer JA, Dijkstra J, Kuik DJ, van den Brekel MW *et al*. Molecular diagnosis of surgical margins and local recurrence in head and neck cancer patients: a prospective study. *Clin Cancer Res* 2004;**10**:3614–20
49 Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM *et al*. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N Engl J Med* 1995;**332**:429–35
50 Barrera JE, Ai H, Pan Z, Meyers AD, Varella-Garcia M. Malignancy detection by molecular cytogenetics in clinically normal mucosa adjacent to head and neck tumors. *Arch Otolaryngol Head Neck Surg* 1998;**124**:847–51

51 Nathan CA, Amirghahri N, Rice C, Abreo FW, Shi R, Stucker FJ. Molecular analysis of surgical margins in head and neck squamous cell carcinoma patients. *Laryngoscope* 2002;**112**:2129–40
52 el-Naggar AK, Hurr K, Batsakis JG, Luna MA, Goepfert H, Huff V. Sequential loss of heterozygosity at microsatellite motifs in preinvasive and invasive head and neck squamous carcinoma. *Cancer Res* 1995;**55**:2656–9
53 Stafford ND. Genetics of Head & Neck Cancer. In: Gleeson MJ, Jones NS, Clarke R, Luxon L, Hibbert J, Watkinson J, eds. *Scott-Brown's Otorhinolaryngology: Head & Neck Surgery*, 7th edn. 2008: Hodder Arnold, London; 2351–8
54 Chang SS, Califano J. Current status of biomarkers in head and neck cancer. *J Surg Oncol* 2008;**97**:640–3
55 Choi HR, Roberts DB, Johnigan RH, Sturgis EM, Rosenthal DI, Weber RS *et al*. Molecular and clinicopathologic comparisons of head and neck squamous carcinoma variants: common and distinctive features of biological significance. *Am J Surg Pathol* 2004;**28**:1299–310
56 Takebayashi S, Hickson A, Ogawa T, Jung KY, Mineta H, Ueda Y *et al*. Loss of chromosome arm 18q with tumor progression in head and neck squamous cancer. *Genes Chromosomes Cancer* 2004;**41**:145–54
57 Coon SW, Savera AT, Zarbo RJ, Benninger MS, Chase GA, Rybicki BA *et al*. Prognostic implications of loss of heterozygosity at 8p21 and 9p21 in head and neck squamous cell carcinoma. *Int J Cancer* 2004;**111**:206–12
58 Yamamoto N, Mizoe J, Numasawa H, Tsujii H, Shibahara T, Noma H. Allelic loss on chromosomes 2q, 3p and 21q: possibly a poor prognostic factor in oral squamous cell carcinoma. *Oral Oncol* 2003;**39**:796–805
59 Bockmuhl U, Ishwad CS, Ferrell RE, Gollin SM. Association of 8p23 deletions with poor survival in head and neck cancer. *Otolaryngol Head Neck Surg* 2001;**124**:451–5
60 Shah SI, Yip L, Greenberg B, Califano JA, Chow J, Eisenberger CF *et al*. Two distinct regions of loss on chromosome arm 4q in primary head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg* 2000; **126**:1073–6
61 Matsuura K, Shiga K, Yokoyama J, Saijo S, Miyagi T, Takasaka T. Loss of heterozygosity of chromosome 9p21 and 7q31 is correlated with high incidence of recurrent tumor in head and neck squamous cell carcinoma. *Anticancer Res* 1998;**18**:453–8
62 Lydiatt WM, Davidson BJ, Schantz SP, Caruana S, Chaganti RS. 9p21 deletion correlates with recurrence in head and neck cancer. *Head Neck* 1998;**20**:113–18
63 Ransom DT, Barnett TC, Bot J, de Boer B, Metcalf C, Davidson JA *et al*. Loss of heterozygosity on chromosome 2q: possibly a poor prognostic factor in head and neck cancer. *Head Neck* 1998;**20**:404–10
64 Pearlstein RP, Benninger MS, Carey TE, Zarbo RJ, Torres FX, Rybicki BA *et al*. Loss of 18q predicts poor survival of patients with squamous cell carcinoma of the head and neck. *Genes Chromosomes Cancer* 1998;**21**:333–9
65 Lee DJ, Koch WM, Yoo G, Lango M, Reed A, Califano J *et al*. Impact of chromosome 14q loss on survival in primary head and neck squamous cell carcinoma. *Clin Cancer Res* 1997;**3**:501–5
66 Scholnick SB, Haughey BH, Sunwoo JB, el-Mofty SK, Baty JD, Piccirillo JF *et al*. Chromosome 8 allelic loss and the outcome of patients with squamous cell carcinoma of the supraglottic larynx. *J Natl Cancer Inst* 1996;**88**:1676–82
67 Li X, Lee NK, Ye YW, Waber PG, Schweitzer C, Cheng QC *et al*. Allelic loss at chromosomes 3p, 8p, 13q, and 17p associated with poor prognosis in head and neck cancer. *J Natl Cancer Inst* 1994;**86**:1524–9

Address for correspondence:
John Greenman,
Medical Research Laboratory,
University of Hull,
Wolfson Building,
Cottingham Road,
Hull, HU6 7RX, UK.

Fax: +44 (0)1482 466996
E-mail: j.greenman@hull.ac.uk

Dr J Greenman takes responsibility for the integrity of the content of the paper.
Competing interests: None declared