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p53 alterations in recurrent squamous cell cancer of the head and neck refractory to radiotherapy

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Summary The aim of the study was to determine the incidence of p53 alterations by mutation, deletion or inactivation by mdm2 or human papillomavirus (HPV) infection in recurrent squamous cell cancer of the head and neck (SCCHN) refractory to radiotherapy. Twenty-two tumours were studied. The p53 status of each tumour was analysed by sequencing of exons 4–9 and by immunohistochemistry. Mdm2 expression was assessed by immunohistochemistry and HPV infection was assessed by polymerase chain reaction of tumour DNA for HPV 16, 18 and 33. Fifteen (68%) of the 22 tumours studied had p53 mutations, while seven had wild-type p53 sequence. p53 immunohistochemistry correlated with the type of mutation. HPV DNA was detected in 8 (36%) tumours and all were of serotype HPV 16. Of these, five were in tumours with mutant p53 and three were in tumours with wild-type p53. Mdm2 overexpression was detected in 11 (50%) tumours. Of these, seven were in tumours with mutant p53 and four were in tumours with wild-type p53. Overall, 21 of the 22 tumours had p53 alterations either by mutation, deletion or inactivation by mdm2 or HPV. In this study, the overall incidence of p53 inactivation in recurrent head and neck cancer was very high at 95%. The main mechanism of inactivation was gene mutation or deletion which occurred in 15 of the 22 tumours studied. In addition, six of the seven tumours with wild-type p53 sequence had either HPV 16 DNA, overexpression of mdm2 or both which suggested that these tumours had p53 inactivation by these mechanisms. This high incidence of p53 dysfunction is one factor which could account for the poor response of these tumours to radiotherapy and chemotherapy. Therefore, new therapies for recurrent SCCHN which either act in a p53 independent pathway, or which restore p53 function may be beneficial in this disease. © 2000 Cancer Research Campaign

Keywords: p53; HPV; mdm2; head and neck cancer

Local recurrence is the most common cause of failure after head and neck cancer surgery and the most important factor which predisposes to recurrence is incomplete surgical resection margins (Snow et al, 1989; Jones et al, 1992). Recurrent squamous cell cancer of the head and neck (SCCHN) causes significant morbidity including effects on speech and swallowing. It is a disease with a very poor prognosis as currently available therapies including re-irradiation (Nickers, 1997) and chemotherapy (Clavel, 1994) all have poor response rates which are short lasting (Forestiere, 1994). The reason for this is unclear but is likely to be multifactorial in nature. One factor which may be important in resistance to therapy is loss of function of the tumour suppressor gene p53. It has been shown that inactivation of the p53 tumour suppressor gene is one of the major predictors of failure to respond to radiotherapy and chemotherapy in many tumour types (McIlwrath et al, 1994; Buttitta et al, 1997; Cutilli et al, 1998). This is because p53 plays a major role in the induction of apoptosis in response to genotoxic agents such as radiotherapy and chemotherapy (Lowe et al, 1993; Huang et al, 1996). Therefore one possible explanation for the poor response rates in recurrent SCCHN to radiotherapy and chemotherapy could be due to a high incidence of p53 inactivation in this disease. No previous studies have been reported on the incidence or mechanism of p53 inactivation in recurrent head and neck cancer. The objectives of this study were therefore to determine the incidence of p53 mutations in this disease and also gain insight into other mechanisms of p53 inactivation such as binding by the cellular protein mdm2 (Oliner et al, 1992; Haupt et al, 1997) and viral protein HPV E6 (Scheffner et al, 1990; Lechner et al, 1992). To determine the incidence of p53 inactivation in recurrent HNSCC, 22 recurrent tumours from patients previously treated with radiotherapy ± surgery were analysed. The p53 status of each tumour was analysed by sequencing and by immunohistochemistry. Tumours were further analysed for mdm2 overexpression by immunohistochemistry and for human papillomavirus (HPV) infection by polymerase chain reaction (PCR) of tumour DNA. Overall we have shown that there is a greater incidence of p53 mutations in recurrent disease compared to primary disease. However, the incidence of HPV infection and mdm2 overexpression was similar to reported studies in primary disease. Nevertheless, we show that the overall incidence of p53 alterations is very high at 95%. This may be one factor which accounts for the poor response of this disease to radiotherapy and chemotherapy and implies new therapies which either restore p53 function or which act in a p53 indpendent manner may prove to be beneficial in this disease.

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METHODS

Tumour collection

Patients with recurrent SCCHN gave consent for biopsies to be taken from the recurrent tumours. Core biopsies were taken using a 14 gauge tru-cut needle under local anaesthetic. One sample was

snap-frozen in liquid nitrogen for DNA extraction. One sample was fixed in phosphate-buffered formalin, embedded in paraffin from which 5-μm sections were cut for immunohistochemical analysis.

Immunohistochemistry

Immunohistochemistry for p53

Immunohistochemistry was carried out on formalin-fixed paraffin-embedded tumours cut into 5-μm sections. Slides were deparaffinized in xylene, hydrated through ethanols 100%, 90%, 70% and then water, then washed in PBS (phosphate-buffered saline). Antigen retrieval was carried out by microwaving in citrate buffer pH 6.0 at 500 W for 25 min and then allowed to cool over 20 min. The slides were washed in PBS for 5 min and then endogenous peroxide activity blocked with 3% v/v hydrogen peroxide in methanol for 10 min. After washing in PBS for 5 min, the slides were blocked with Universal blocking solution (Biogenex) for 10 min, and then primary antibody (DO-1 Oncogene Science) at a dilution of 1/1000 in Dako antibody diluent solution, added for 1 h at room temperature. Antigen detection was done using a biotinylated second antibody followed by streptavidin as supplied in the Biogenex link/label kit. The chromogen used for detection was diaminobenzidine (Vector) for 3–10 min. The sections were counterstained with haematoxylin, dehydrated in graded alcohols followed by xylene, and then mounted in DPX mounting medium (BDH Chemicals). The immunohistochemistry pattern was scored using a histoscore based on intensity of staining and percentage of positive cells staining and given a score out of a maximum of 6 (Table 1).

Table 1

Intensity of staining	Score	Cells staining positive (%)	Score
None	0	0	0
Mild	1	5–20	1
Moderate	2	20–80	2
Severe	3	> 80	3

Immunohistochemistry for mdm2

The protocol used was as described for p53 immunohistochemistry. The primary antibody used was a mouse monoclonal antibody SMP14 (Dako) at a dilution of 1/50 in PBS. Mdm2 staining was then scored by the % of cells staining positive and by the intensity of staining as described above.

DNA extraction

Genomic DNA was isolated directly from frozen tumours by lysis buffer containing 10 mM Tris–HCl, 10 mM EDTA, 10 mM sodium chloride, 4% N-lauryl sarcosine and 2.75 mg ml^{-1} proteinase K, followed by overnight digestion at 55°C and EtOH precipitation. The resulting pellet was washed with 70% EtOH, dried, and resuspended in 100 μl TE and stored at 4°C.

p53 sequencing

Exons 4 to 9 were sequenced using DNA extracted from pre-treatment tumour biopsies. This was done by Oncormed Corporation, Gaithersburg, Maryland.

HPV analysis

The presence of HPV in tumour DNA was assessed by PCR. The primers for the amplification of HPV 16 and HPV 18 have been described previously (Yeudall et al, 1991) and amplify fragments of the E6/E7 (HPV 16) or E6 regions (HPV 18), which are 165 and 99 bp respectively. HPV 33 primers were those as described by Haraf et al (1996).

Table 2 Primary tumour, site of recurrence and previous treatment

Pt no.	Sex	Age	Primary	Site of recurrence	Prior therapy		
					Chemotherapy	Radiotherapy	Surgery
1	M	61	Piriform fossa	Left cervical	Yes	Yes	No
2	M	74	Hypopharynx	Bilateral cervical	Yes	Yes	No
3	M	32	Tongue	Right submandibular	No	Yes	Yes
4	F	80	Palate	Right clavicular area	No	Yes	Yes
5	F	68	Floor of mouth	Right neck-post triangle	No	Yes	Yes
6	F	38	Left auditory canal	Left cervical	Yes	Yes	No
7	M	53	Left temple	Left preauricular area	No	Yes	Yes
8	F	72	Right retromolar trigone	Right cervical	No	Yes	No
9	M	56	Floor of mouth	Left submandibular	No	Yes	Yes
10	M	88	Left palatoglossal fold	Bilateral cervical	No	Yes	Yes
11	M	76	Supraglottic larynx	Left cervical	No	Yes	No
12	M	82	Retromolar trigone	Left cervical	No	Yes	No
13	M	62	Tongue	Left cervical	No	Yes	Yes
14	M	75	Supraglottic larynx	Left submandibular	No	Yes	Yes
15	M	60	Larynx	Right cervical	No	Yes	Yes
16	F	64	Piriform fossa	Right cervical	Yes	Yes	No
17	M	59	Oropharynx	Left clavicular	No	Yes	Yes
18	F	58	Supraglottic larynx	Left cervical	No	Yes	Yes
19	F	59	Oropharynx	Left cervical	No	Yes	Yes
20	M	51	Tongue	Right cervical	No	Yes	Yes
21	F	75	Post cricoid	Left cervical	No	Yes	No
22	M	65	Supraglottic larynx	Left supraclavicular	No	Yes	Yes

The HPV16 primers were: 5′-TTAATTAGGTGTATTAACTG-3′ and 5′-TGCATGATTACAGCRGGGTT-3′. The HPV18 primers were: 5′-ATCTGTGTGCACGGAACTAAC-3′ and 5′-AATGCA-AATTCAAATACCTC-3′. The HPV33 primers were: 5′-GTGC-CAAGCATTGGAGACAA-3′ and 5′-GATAAGAACCGCAAAC -ACAG-3′.

SiHa DNA (single copy HPV 16) and HeLa DNA (HPV 18) were used as positive controls. HPV amplification was carried out at 95°C for 3 min, then 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, followed by a final extension time of 10 min at 72°C. An aliquot was run on a 1% agarose gel (Nu-Sieve) containing 0.5 μg ml^{-1} ethidium bromide in TBE buffer and then photographed under UV light to check for correct amplification.

Table 3 p53 sequence of recurrent squamous cell cancers studied

Pt no.	Exon	Codon	Base-pair change	Amino acid change	p53 Gene sequencing (Exons 4–9)
1	5	376	Splice site mutation	Truncated protein	Mutant-non-sense
2	8	273	CGT to TGT	Arginine to cysteine	Mutant-mis-sense
3	8	307	Deletion G	Stop signal	Mutant-non-sense
4	6		51 splice site mutn AG to AA	Truncated protein	Mutant-non-sense
5	5	141	TGC to TAC	Cysteine to tyrosine	Mutant-mis-sense
6	9	336	Frameshift mutation	Stop signal	Mutant-non-sense
7	8	280	AGA to ATA	Arginine to isoleucine	Mutant-mis-sense
8	8	282	CGG to TGG	Arginine to tryptophan	Mutant-mis-sense
9	wt				Wild type
10	wt				Wild type
11	wt				Wild type
12	8	266	GGA to TGA	Glycine to stop signal-truncated protein	Mutant-non-sense
13	6	205	TAT to TGT	Tyrosine to cysteine	Mutant-mis-sense
14	wt				Wild type
15	7	233	11 bp insertion	Stop signal at codon 250	Mutant-non-sense
16	9	317	CAG to TAG	Glutamine to stop signal-truncated protein	Mutant-non-sense
17	wt				Wild type
18	6	222	Deletion G	Stop signal	Mutant-non-sense
19	wt				Wild type
20	5	163	TAC to TGC	Tyrosine to cysteine	Mutant-mis-sense
21	wt				Wild type
22	5	167	10 bp deletion	Stop signal	Mutant-non-sense

Of 22 tumours studied, 15 had mutant p53 and 7 had wild type p53. Of the mutant p53 sequences, 6 were missense mutations and 9 were nonsense mutations.

Table 4 p53 and mdm2 immunohistochemistry histoscores and HPV PCR analysis in squamous cell cancers studied

Patient no.	p53 gene seq.	p53 IHC	p53 histoscore	mdm2 IHC	mdm2 histoscore	HPV16	HPV18/33
1	Mutant-mis-sense	–	0	+	2	–	–
2	Mutant-non-sense	+	4	–	0	–	–
3	Mutant-non-sense	–	0	–	0	+	–
4	Mutant-mis-sense	–	0	+	2	–	–
5	Mutant-non-sense	+	6	–	0	–	–
6	Mutant-mis-sense	+	6	+	4	–	–
7	Mutant-mis-sense	+	6	–	0	–	–
8	Mutant-mis-sense	+	5	+	2	–	–
9	Wild type	+	5	+	3	–	–
10	Wild type	–	0	–	0	+	–
11	Wild type	–	0	+	5	–	–
12	Mutant-non-sense	–	0	+	3	+	–
13	Mutant-mis-sense	+	4	–	0	+	–
14	Wild type	+	2	–	0	–	–
15	Mutant-non-sense	–	0	–	0	+	–
16	Mutant-non-sense	–	0	–	0	+	–
17	Wild type	+	2	+	5	+	–
18	Mutant-non-sense	–	0	+	4	–	–
19	Wild type	–	0	–	0	+	–
20	Mutant-mis-sense	+	4	–	0	–	–
21	Wild type	+	6	+	5	–	–
22	Mutant-non-sense	–	0	+	4	–	–

p53 immunohistochemistry histoscore correlated with the type of gene sequence; non-sense mutations had low histoscores and mis-sense mutations had high histoscores. Mdm2 protein expression was detected in 11 tumours and HPV16 detected in eight tumours. HPV18 and 33 DNA were not detected in any tumour.

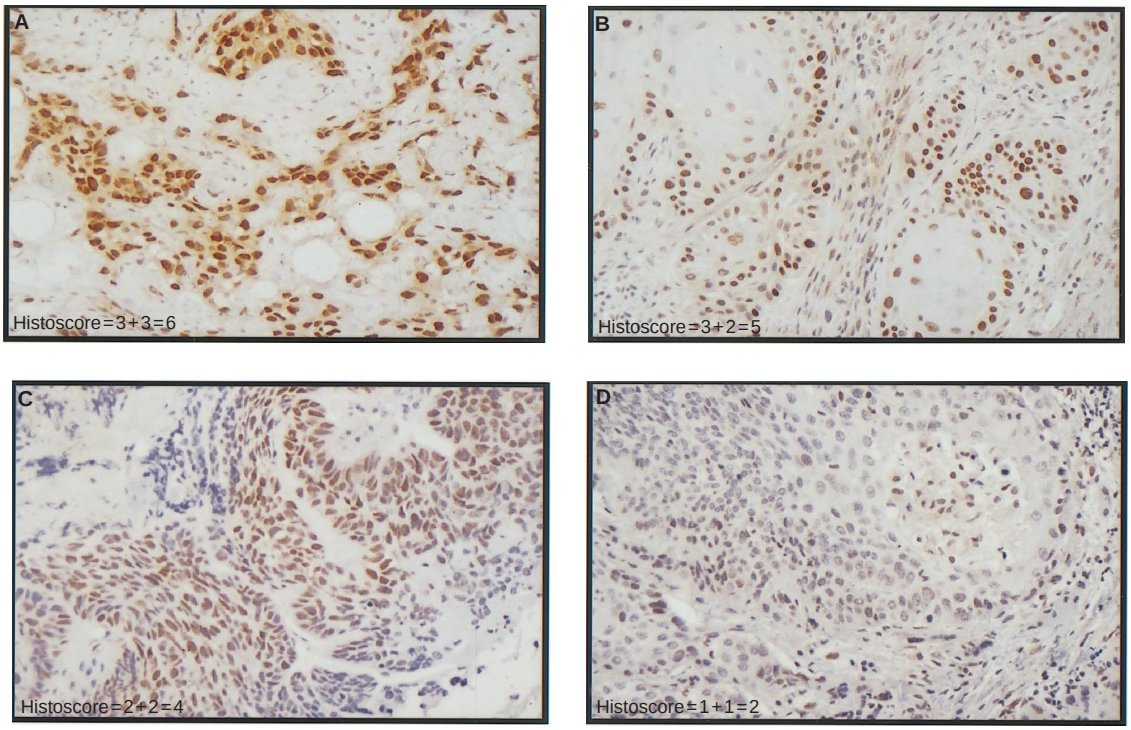

Figure 1 Examples of p53 immunohistochemistry staining in SCCHN biopsies. Staining was graded using a histoscore grading system based on the intensity of staining and the percentage of cells staining positively as described in Methods

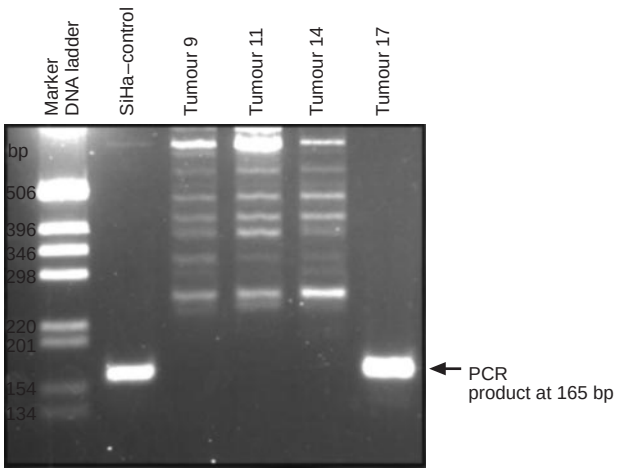

Figure 2 HPV 16 E6/7 analysis of tumour DNA by PCR. DNA from SiHa cell line was used as a positive control for HPV16 E6/7. Tumour 17 is positive for HPV 16 with the correct PCR product at 165 base pairs

RESULTS

Primary tumour, site of recurrence and previous treatment

The primary tumour, site of recurrence and previous therapy for each patient studied are shown in Table 2. The mean age was 64 and the male to female ratio was 14:8. All patients had received radiotherapy. In addition, four patients had received chemotherapy and 14 patients had had surgery. The site of tumour recurrence was most commonly regional in the site of draining lymph nodes.

Incidence of p53 mutation in recurrent SCCHN is high and type of mutation correlates with immunohistochemistry

The p53 sequence and immunohistochemistry for each tumour is shown in Tables 3 and 4. Of the 22 tumours studied, 15 had mutations in the *p53* gene of which six were mis-sense mutations and nine were non-sense mutations. Generally, the immunohistochemistry pattern correlated directly with the type of p53 mutation, i.e. mis-sense mutations gave high scores and non-sense mutations gave low scores. One patient (patient 6) had a non-sense mutation yet stained positively by immunohistochemistry. The site of the mutation in this patient's *p53* gene was in codon 9 and this would account for its detection by immunohistochemistry using the DO-1 antibody. Of the seven patients with wild-type p53 sequence, five were negative on immunohistochemistry (tumours 10, 11, 14, 17, 19) and two were positive indicating overexpression of wild-type p53 (tumours 9, 21). Examples of p53 immunohistochemistry with the relevant histoscores is shown in Figure 1.

Incidence of HPV infection and mdm2 overexpression

Eight tumours were positive for HPV DNA, all of which were serotype HPV 16. Of these, five were in tumours with mutant p53 and three were in tumours with wild-type p53. A typical positive PCR reaction for HPV 16 is shown in Figure 2.

Mdm2 expression was detected in 11 of the 22 tumours (50%). Of these, five were weakly positive (histoscore = 2, 3) and six were strongly positive (histoscore = 4, 5, 6). Mdm2 expression was present in seven tumours with mutant p53 and in four tumours with wild-type p53.

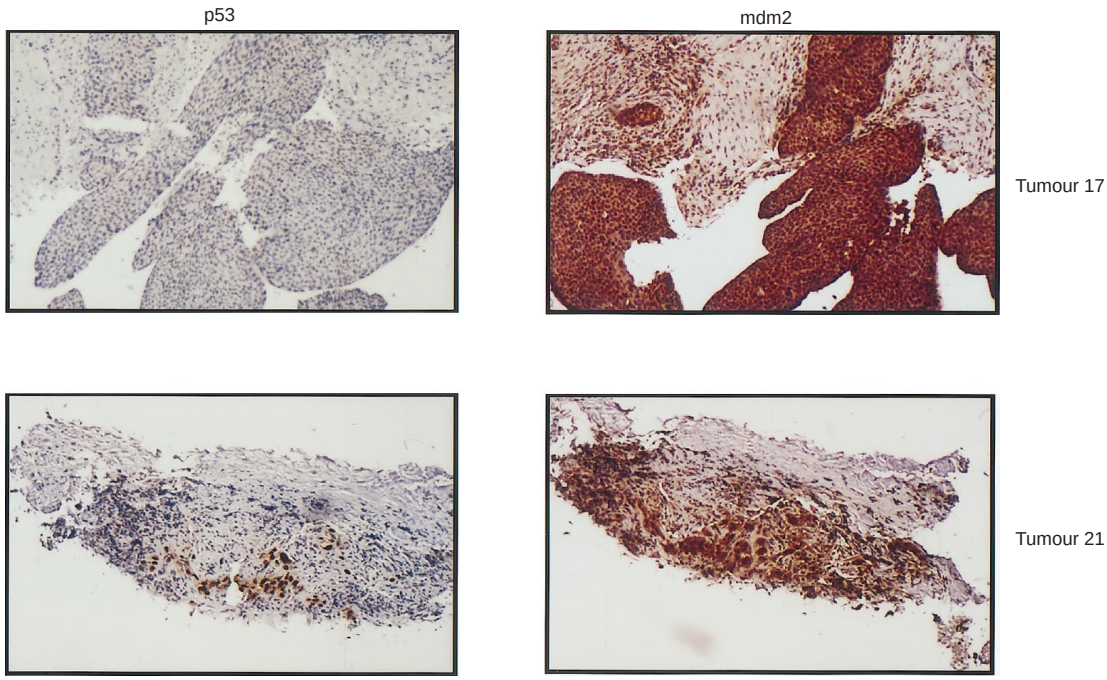

Figure 3 Examples of p53 and mdm2 immunohistochemistry in tumours with wild-type p53 gene sequence. Tumour 17 has weak p53 staining but intense mdm2 staining suggesting p53 inactivation by mdm2 overexpression. Tumour 21 has overexpression of wild type p53 and also intense mdm2 staining suggesting p53 may be functional in this tumour but inactivated by high expression of mdm2

Of the five tumours with wild-type p53 sequence and negative or weakly positive p53 immunohistochemistry, two had HPV 16 expression (tumours 10, 19), one had high expression of mdm2 (tumour 11), one tumour had both (tumour 17) and one had neither. Of the two tumours with wild-type p53 and strongly positive p53 immunohistochemistry (tumours 9, 21), both had high expression of mdm2 indicating inactivation by mdm2. Thus, six of the seven tumours with wild-type p53 had inactivation of p53 either by HPV 16 infection or by overexpression of mdm2. Examples of mdm2 immunohistochemistry in tumours 17 and 21 are shown in Figure 3.

DISCUSSION

In this paper we have shown that in recurrent SCCHN there is a high incidence of p53 mutation. Fifteen of the 22 tumours (68%) had either a p53 mutation or deletion. Of these, six were mis-sense mutations, whereas nine had non-sense inactivation. The immunohistochemistry results correlated very well with the mutations seen. Previous studies in primary head and neck cancer have shown an incidence of p53 mutation of 38–53% (Brachmann et al, 1992; Boyle et al, 1993; Mao et al, 1996; Olshan et al, 1997; Koh et al, 1998). It has been suggested that p53 mutation is an early event in head and neck carcinogenesis. Boyle et al (1993) showed an incidence of mutation of 19% in pre-invasive lesions increasing to 43% in invasive lesions. However, Chung et al (1993) showed that the frequency of p53 mutations did not increase with stage of disease with a reported incidence of 47% in stage I/II disease and 37% in stage III/IV disease. In our study, we have reported an incidence of p53 mutation in recurrent SCCHN of 68%, which is higher than that reported for primary disease. We did not carry out microdissection of tumour cells from surrounding normal cells in the preparation of tumour DNA so it is possible that some tumours with wild-type *p53* gene sequence are in fact false-negatives and therefore the incidence may be higher than 68%. Our figures (and those reported for primary disease by other studies) are also based upon sequencing exons 4–9 of the *p53* gene. Although 95% of mutations occur in these exons, some mutations occur in intron regions and also other exons. Therefore the incidence of p53 mutations in our study may be higher. Similarly, the incidence in previously published studies on primary head and neck cancer may also be slightly higher than 38–53%. It is also possible that previous studies in primary disease have underestimated the incidence of p53 mutation due to the heterozygous nature of primary tumours resulting in false-negative results on gene sequencing. In recurrent tumours, clonal expansion of tumour cells refractory to radiotherapy occurs and therefore the pickup rate for detecting p53 mutation may be more accurate. However, another possibility may be the induction of p53 mutations by radiotherapy treatment itself since radiation is a potent DNA damaging agent and induces single and multiple base pair deletions in DNA. It is difficult to distinguish between these two possibilities but one method could be to prepare molecular probes to the p53 mutation in each recurrent tumour and then to use these probes to screen the primary tumours to look for the mutation. This study is currently underway and will be reported separately.

p53 can be inactivated by HPV due to the ability of HPV E6 protein to bind to and promote the degradation of the product of the *p53* gene (Levine, 1990; zur Hausen, 1994). Indeed, inactivation of p53 by HPV E6 is the major mechanism of p53 inactivation in cervical carcinogenesis (zur Hausen, 1994) with approximately 80–90% of cervical cancers containing HPV DNA (Yoshikawa, 1991). Previous studies have shown HPV DNA in tumours of the

head and neck but its prevalence varies from 10–58% (Haraf et al, 1996; Paz et al, 1997; Koh et al, 1998; Miguel et al, 1998). A metanalysis by McKaig et al (1998) showed an overall incidence of 34.5% with the predominant type being HPV 16. The incidence also varies with tumour site with the highest prevalence being in tonsillar carcinoma (Paz et al, 1997). In our study in recurrent tumours of the head and neck, we found an overall incidence of 36% (8/22), all of which were HPV 16. These results were very similar to the meta-analysis figures by McKaig for primary tumours and this would suggest that HPV infection is not a major aetiological factor in recurrent disease. One of the limitations of our study, and of previously published studies, is that HPV detection is based upon PCR analysis of HPV DNA and not mRNA of HPV E6–E7 regions. It is possible that PCR analysis of HPV DNA can overestimate the presence of HPV due to cross-contamination resulting in false-positives. Since RNA is readily degradable, cross-contamination is less likely to result in false-positives. Another advantage of using RNA is that it proves that any HPV present in tumour cells is actually expressed at the mRNA level. Some studies have reported HPV mRNA detection using in situ hybridization of paraffin-embedded samples (Stoler et al, 1992). A more accurate method, however, is to use reverse transcription PCR (RT-PCR) as reported by Czegledy et al (1995). In our study, DNA was used because the tumour samples were too small to allow an adequate amount of RNA to be prepared. Of the eight tumours which were positive for HPV, five were in tumours with mutant p53 and three were in tumours with wild-type p53. These figures are similar to those in primary tumours as reported by Koh et al (1998) and suggests that p53 mutation and HPV positivity are not mutually exclusive mechanisms of p53 inactivation. This is in contrast to cervical carcinogenesis where p53 mutations are rare in cases with HPV but common in malignancies devoid of HPV infection (Park et al, 1990; Crook et al, 1991).

Another factor which can inactivate p53 is the cellular protein mdm2. Mdm2 inhibits the transcriptional activity of p53 by binding to the N terminus of p53 (Haupt et al, 1997) which in turn leads to the degradation of p53 via the ubiquitin degradation pathway. Thus amplification or overexpression of mdm2 can cause p53 inactivation (Oliner et al, 1992). In primary SCCHN, mdm2 overexpression is reported to be 40% (Matsumura et al, 1996). Girod et al (1995) showed an increase in mdm2 expression from premalignant to malignant lesions indicating that this may be a mechanism for inactivation of p53 early in head and neck carcinogenesis. Further evidence to suggest this was reported by Pruneri et al (1997), who showed that out of 17 laryngeal tumours overexpressing mdm2, ten of these also overexpressed p53 and all ten had wild-type p53 sequence. The importance of mdm2 overexpression in recurrent SCCHN is unknown. In the small group of tumours which we have studied, we have shown an incidence of 50% of which 5/11 were weakly positive and 6/11 were strongly positive for mdm2. Mdm2 overexpression was present in seven tumours with mutant p53 and in four tumours with wild-type p53, and this also suggests that p53 mutation and mdm2 overexpression are not mutually exclusive mechanisms of p53 inactivation. A particularly intriguing observation is that of the seven tumours which had wild-type p53 sequence, six were positive for either HPV infection, mdm2 overexpression or both. Thus in wild-type p53 tumours, the *p53* gene is inactivated by other mechanisms making the overall incidence of p53 inactivation in recurrent SCCHN in this study to be 95% (21 out of 22 tumours). It is unclear whether or not this overall incidence is greater than primary SCCHN since no study has ever examined p53 mutation, HPV infection and mdm2 overexpression in the same group of tumours. The observation that the incidence of HPV infection and mdm2 overexpression are similar to primary disease indicates that these factors have a limited role in the aetiology of recurrent SCCHN but may be more important in tumours in which the *p53* gene is not inactivated by mutation. We have shown that the incidence of p53 mutation in recurrent disease is higher than primary disease and have suggested that one possible reason for this could be due to DNA damage induced by radiation itself. We are currently carrying out a study to determine the importance of this observation using molecular probes to each individual p53 mutation to screen the primary tumour for the mutation.

The observation that recurrent disease has a very high incidence of p53 inactivation is clearly important as p53 is central to apoptosis induced by radiation and many chemotherapeutic agents. This may account for the poor response of these tumours to both reirradiation and chemotherapy. Therefore, new therapies which can restore p53 functionality, such as adenoviral mediated transfer of wild-type p53 (Roth et al, 1996), may be beneficial in this disease. Indeed, in vitro and in vivo studies using such a technique have demonstrated growth suppression in SCCHN cell lines (Liu et al, 1994, 1995; Clayman et al, 1995). Results of a phase I study in patients with recurrent SCCHN were also recently reported (Clayman et al, 1998) and this showed evidence of tumour necrosis in some patients. Other methods of restoring p53 function could be to use small molecules to inactivate the mdm2 protein (Bottger et al, 1998), or drugs which interfere with HPV E6 binding to wild-type p53 thus restoring function of p53. Recently, the selectively replicating adenovirus, Onyx-015, which targets cells with mutant p53, was reported (Bischoff et al, 1996; Heise et al, 1997). Results of a phase I study in patients with recurrent head and neck cancer using this virus appear to be promising (Ganly et al, 1997). Other approaches to the treatment of this disease may be to use drugs which act in a p53-independent manner. For example, Taxanes (Taxol) act by inducing p53-independent apoptosis and have been successful in the treatment of refractory cisplatin-resistant ovarian cancer, a disease where there is also a high incidence of p53 inactivation (Righetti et al, 1996; Buttitta et al, 1997). Catimel et al (1994) has shown this agent to be effective in recurrent SCCHN but there is no evidence yet that it has increased benefit over cisplatin–5-fluorouracil chemotherapy. However, newer agents which act in a p53-independent manner may prove to be more beneficial.

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