Virchows Arch (1995) 427:153–157

ORIGINAL ARTICLE

Malte Barten · Christiane Ostwald
Karin Milde-Langosch · Petra Müller
Yvonne Wukasch · Thomas Löning

HPV DNA and p53 alterations in oropharyngeal carcinomas

Received: 24 March 1995 / Accepted: 29 June 1995

Abstract We have examined a series of 37 oropharyngeal squamous cell carcinomas for the presence of HPV 6/11, 16, and 18 DNA by polymerase chain reaction (PCR)/Southern blotting and for p53 alterations by immunohistochemistry and mutation screening with temperature gradient gel electrophoresis (TGGE). HPV sequences were found in a total of 26 of 37 cancers (70.3%), most frequently HPV 16 (20/37) followed by HPV 18 (11/37). Double infections with HPV 16 and 18 were present in 5 tumours. p53 accumulation was detectable immunohistochemically in 21 of 37 carcinomas (56.8%). There were remarkable differences in the distribution of immunoreactive tumour cells in relation to the tumour grade. A mutation screening for p53 by TGGE, directed to the amplified exons 5–8, revealed p53 mutations in 14 of 37 carcinomas (37.8%). Mutations in two different exons were present in 3 tumours, 11 tumours being hit once. Exon 7 was mutated in 6 carcinomas, exons 5 and 8 in 4 cases, and exon 6 in 3 cases. When grouping the tumours with p53 mutation according to their HPV state, HPV-positive cases showed slightly more mutations (11/26) than HPV-negative cases (3/11). Only 5 of 37 carcinomas (13.5%) contained neither HPV DNA nor p53 alterations. Our results indicate that high-risk HPV and p53 mutations frequently coexist in oropharyngeal carcinomas, in contrast to genital tumours, notably carcinomas of the cervix uteri. This may reflect different pathways in carcinogenesis in squamous cell epithelium from different sites.

M. Barten (✉) · Ch. Ostwald · Y. Wukasch
Department of Pathology, University of Rostock, Strempelstrasse 14, D-18055 Rostock, Germany

P. Müller
Department of Internal Medicine, University of Rostock, Germany

Th. Löning · K. Milde-Langosch
Department of Gynaecological Histopathology and Electron Microscopy, University of Hamburg, Germany

Key words Papillomavirus · p53 · Oropharyngeal carcinoma · Polymerase chain reaction · Temperature gradient gel electrophoresis

Introduction

Smoking and high alcohol consumption are well-known risk factors for oropharyngeal cancer [2, 19, 26]. Possible involvement of viruses in the induction of these tumours has been discussed for many years and in the last decade viral factors have attracted further interest when human papillomaviruses (HPV) detected in oropharyngeal tumours with increasing frequency, especially using polymerase chain reaction (PCR)-based DNA detection techniques [27, 29, 37, 39]. Now it seems well established that more than half of the oral carcinomas contain HPV DNA, predominantly of high-risk types.

The possible oncogenic actions of HPV have mainly been investigated in carcinomas of the cervix uteri and their cell lines. It was found that the continuing expression of the E6/E7 ORF of high-risk HPV was necessary for the maintenance of the malignant phenotype and tumourigenicity of cervical carcinoma cell lines [41]. These viral proteins can exert their oncogenic potential by complexing and inactivating the products of the retinoblastoma tumour suppressor gene (Rb) and the p53 gene [21, 33, 38]. This results in the disruption of the cell cycle guardian function of these genes and allows cell cycle progression following DNA damage [18].

Mutation of a tumour suppressor gene and loss of the wild-type allele is another pathway of abrogating natural tumour suppressor functions. Mutations in the tumour suppressor gene p53 are frequently found and operate in a dominant manner in many cancers of different histogenesis [20], but it is very rare in HPV-positive squamous cell carcinomas of the cervix uteri [10, 11, 16, 22, 24, 33, 38]. The present state of knowledge is even suggestive of an inverse relationship between HPV infection and p53 mutations in these tumours. It is thought that p53 inactivation in HPV-positive cervical squamous cell

carcinomas is caused by interaction with HPV E6 oncogen products and this may lead to an accumulation of additional genetic abnormalities, which could trigger, together with host factors, the multistep process of carcinogenesis [41].

In the present study we investigated the frequency and coexistence of HPV DNA of the types 6/11, 16, and 18 and p53 alterations in oropharyngeal carcinomas. HPV detection was done by PCR/Southern blotting. p53 alterations were studied by immunocytochemistry and mutation screening with temperature gradient gel electrophoresis (TGGE).

Materials and methods

Tissue specimens were obtained immediately after surgical removal or excision of oropharyngeal cancer. Unfixed specimens for DNA preparation were frozen and stored in liquid nitrogen. For immunohistochemical staining formalin-fixed and paraffin-embedded tissue specimens were used. Tissue from HPV-positive carcinomas of the cervix uteri served as positive controls for HPV analysis. Samples of the tumour cell lines A 431 (mutated in exon 8) and CEM (mutated in exon 5 and 7) and DNA from a squamous cell carcinoma (mutated in exon 6) were used as positive controls in the p53 mutation screening.

For immunohistochemical p53 detection 4-µm thick sections were cut from paraffin-embedded tumour samples, dewaxed in xylene and rehydrated in a series of alcohols. A pretreatment for antigen retrieval was done by microwave heating in citrate buffer (pH 6.0) for 15 min at 900 W. Endogenous peroxidase was inhibited with 1% hydrogen peroxide and nonspecific binding blocked with normal rabbit serum. Sections were incubated with the mouse monoclonal p53 antibody DO1 (Ab-6, Oncogene Science, Uniondale, N.Y.) detecting mutated and wt p53, at a dilution of 1:100 for 60 min. The antibody DO1 reacts with an epitope between amino acids 37 and 45 located near the amino terminus of p53. After washing with PBS, sections were incubated for 30 min with biotinylated rabbit antimouse IgG (Dianova, Hamburg, Germany) and incubated with avidin biotin peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, Calif.) for 30 min. The enzyme reaction was developed for 5 min with diaminobenzidine tetrahydrochloride diluted in hydrogen peroxide substrate. Finally the sections were counterstained with haematoxylin, dehydrated and mounted in Pertec (Medite, Burgdorf, Germany). Omission of primary antibody was used as negative and a p53 immunoreactive squamous cell carcinoma as positive control. The percentage of positively stained nuclei was calculated by counting 500 nuclei in each slide. A semiquantitative grading system was used, with the following criteria: – no immunoreaction, + less than 10% of tumour cells stained, ++ 10–30% of tumour cells stained, +++ more than 30% of tumour cells stained.

The procedure of DNA extraction was described in detail previously [29]. High molecular weight DNA from tissue samples was prepared by digestion with a solution of 0.01 M TRIS-HCl/0.005 M EDTA (pH 7.8) containing 1% *N*-lauroylsarcosin, 500 µg/ml proteinase-K and 400 µg/ml RNAse. The DNA was extracted with phenol/chloroform/isoamylalcohol (50:49:1), precipitated by ethanol, air dried, redissolved in 0.01 M TRIS-HCl/0.001 M EDTA (pH 8.0), and characterized by ultraviolet spectrophotometry and agarose gel electrophoresis.

HPV DNA was detected by PCR using primers specific for HPV 6/11 [12], HPV 16 [37], and HPV 18 [35]. The amplification of HPV target sequences was carried out in 25 µl of a reaction mixture containing 0.5–1.0 µg of genomic DNA, 50 mmol/l KCl, 10 mmol/l TRIS-HCl (pH 8.3), 1.5 mmol/l $MgCl_2$, 200 µmol/l of each nucleotide dATP, dCTP, dGTP and dTTP, 0.5 µmol/l of each primer and 1 U of Taq DNA polymerase (Perkin Elmer, Überlingen, Germany). The samples were subjected to 40 cycles of amplification under the following conditions: denaturation for 1 min at 94°C, annealing for 2 min at 40°C for HPV 6/11, 30 s at 64°C for HPV 16, 2 min at 45°C for HPV 18, and extension for 2 min at 72°C. The cycling procedure was preceeded by 5 min at 94°C and finished by an additional extension for 3 min at 72°C. The products were analysed on 2% agarose gel, Southern blotted and hybridized with digoxigenin-labelled type-specific probes for HPV 6/11 [12], HPV 16 [37], HPV 18 [35]. The hybrids were detected by chemiluminescence using a nucleic acid detection assay (Boehringer, Mannheim, Germany). To prove the presence of amplificable DNA in the DNA extractions, all of them were amplified with primers for the human betaglobin gene.

The procedure of screening for p53 mutations by TGGE was described in detail by Scholz et al. [34]. DNA of the exons 5, 6, 7, and 8 were amplified separately by PCR using the following primers: forward (GC40) nt 13040–13059, reverse nt 13281–13261 (exon 5), forward (GC40) nt 13261–13281, reverse nt 13447–13428 (exon 6), forward (GC40) nt 13992–14013, reverse nt 14125–14105 (exon 7), and forward nt 14438–14459, reverse (GC40) nt 14612–14591 (exon 8). The PCR was performed using 50 µl of a reaction mixture containing 0.5 µg of genomic DNA, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 10 mmol/l TRIS-HCl (pH 8.3), 100 µmol/l of each nucleotide dATP, dCTP, dGTP and dTTP, 10 pmol of each primer, and 1 U of Taq DNA polymerase (Perkin Elmer). Samples were subjected to 40 cycles of amplification, using the following cycling programs: denaturation for 30 s at 94°C, annealing for 30 s at 55°C for exon 5 and 8 or 60°C for exon 6 and 7, and 1 min extension at 72°C. To test the efficacy of these procedures 10 µl of the PCR products was electrophoresed on 2% agarose gel; then the PCR products were purified by extraction with phenol/chloroform/isoamylalcohol (25:24:1), precipitated with ethanol, and redissolved in 10–30 µl TGGE running buffer (20 mM MOPS, 1 mM EDTA pH 8.0). Five microlitres of the purified PCR products mixed with 0.5 µl dye solution containing 0.5 mg/ml bromphenol blue and 0.5 mg/ml xylencyanol FF were taken for TGGE, using the TGGE system from Diagen (Hilden, Germany). The probes were loaded into a 8% polyacrylamide gel (8 M urea, 20 mM MOPS, 1 mM EDTA pH 8.0, and 2% glycerol) after a prerun of 30 min at 250 V and 20°C to equilibrate the system. The TGGE was performed under the following conditions: run-in time (20°C) 15 min for exon 5, 100 min for exon 6, 30 min for exon 7 and 180 min for exon 8, temperature gradient 55–75°C (exon 5), 35–68°C (exon 6), 40–75°C (exon 7) and 40–78°C (exon 8), running time 3 h 45 min (exon 5), 2 h 50 min (exon 6), 2 h 30 min (exon 7), and 3 h (exon 8). Resulting DNA bands were visualized by silver staining (Diagen).

Results

Our study included 33 oral and 4 pharyngeal carcinomas, all of which were classified histopathologically as squamous cell carcinomas. Twenty-nine patients were male and 8 were female, with a mean age of 58 years (range, 39–88). Most frequently G2 tumours were found (22/37). Thirteen tumours were G1 and 2 were G3. One oral tumour dedifferentiated within 1 year to a spindle cell carcinoma. The tumours were preferentially localized in the floor of the mouth (14/37), followed by the lower lip (7 cases), buccal region (5 cases), tongue (4 cases), palate (3 cases), and hypopharynx (4 cases). According to size and local extension 11 tumours were stage pT1, 12 were pT2, 5 were T3/pT3, and 9 were T4/pT4.

HPV DNA of the high-risk types 16 and 18 were detected in 26 out of 37 oropharyngeal carcinomas (70.3%) with PCR/Southern blot analysis. HPV 16 was present in 20 (54.1%) and HPV 18 in 11 tumours (29.7%), of which 5 tumours were positive for both HPV 16 and 18.

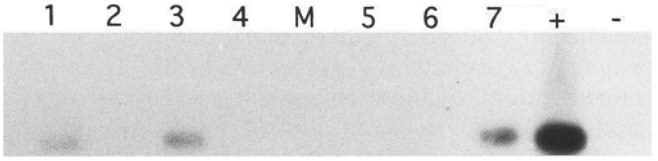

Fig. 1 Southern blot analysis of HPV 16 polymerase chain reaction (PCR) products visualized by chemiluminescence. HPV 16 positive carcinomas in *lanes 1, 3, and 7*; -, negative control; +, positive control (squamous cell carcinoma of the cervix uteri); *M* molecular size marker

Table 1 HPV DNA detection with PCR/Southern blot analysis in oropharyngeal squamous cell carcinomas

HPV negative	HPV positive				
	16	18	16 and 18	16 and 6/11	Total
11 (29.7%)	14	6	5	1	26 (70.3%)

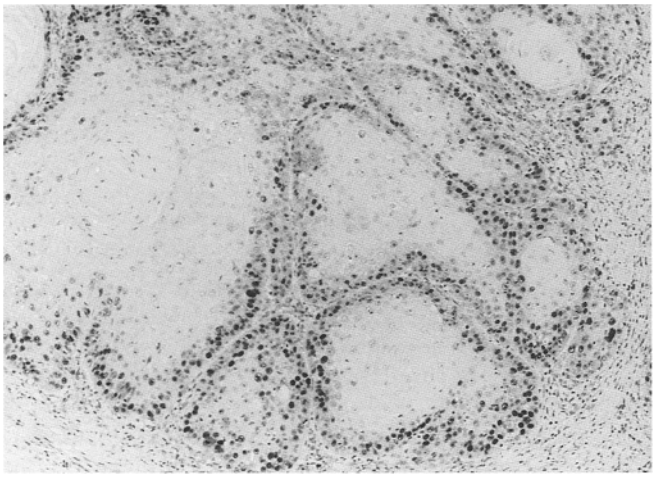

Fig. 2 Well differentiated oral squamous cell carcinoma with p53 immunoreactivity of the outer layer of the tumor cell aggregates. (ABC peroxidase, ×120)

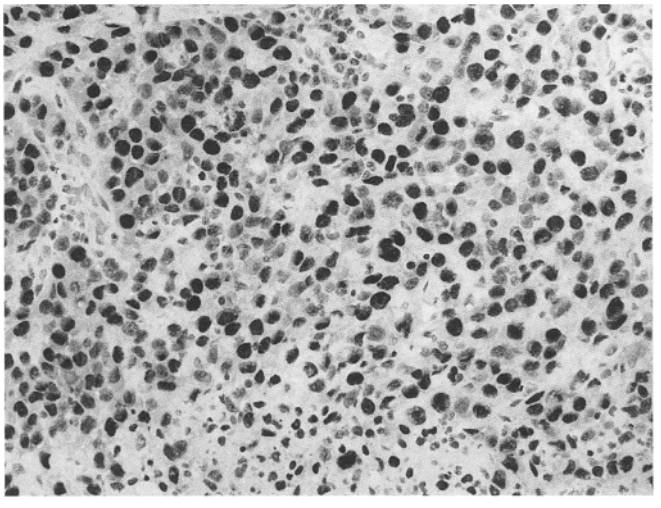

Fig. 3 Poorly differentiated oral squamous cell carcinoma with diffuse nuclear p53 immunoreactivity. (ABC peroxidase, ×320)

One tumour harboured both HPV 6/11 and 16. We found similar rates of HPV-positive and negative cases in different regions of the oral cavity and pharynx, with the exception of the buccal region where all examined carcinomas were HPV positive. There were no clear correlations between HPV status, histopathological tumour stage, and grade.

Immunohistochemically detectable amounts of p53 protein in an intranuclear position were present in 21 of 37 tumours (56,8%). The immunostaining was estimated as ++ in 9 tumours, +++ in 8, and + in 4. G1 tumours displayed a typical zonal distribution of immunoreactivity. The outer layers of tumour cell nests were positive and the central higher differentiated parts negative. In contrast p53-positive G3 carcinomas showed a diffuse distribution of immunoreactive tumour cells and in G2 carcinomas an intermediate pattern was present. According to these tumour grade related differences high numbers of p53 positive tumour cells were detectable only in G2 and G3 carcinomas. Immunoreactivity for p53 was found in 15 of 26 HPV-positive carcinomas and 6 of 11 HPV-negative cases. There was no clear correlation of p53 immunoreactivity to pT/T stage and tumour site.

A p53 mutation results in the occurrence of 4 bands in the TGGE due to formation of 2 homoduplices and 2 heteroduplices by amplification, denaturation, and renaturation of both mutated and nonmutated p53 allelle. This pattern was present in 14 of 37 carcinomas (37.8%). In 11 carcinomas one exon was mutated and two exons were altered in 3 tumours. Most frequently exon 7 was involved (6) followed by exon 5 and 6 (4), and exon 8 (3). In 2 of 14 carcinomas with p53 mutation no p53 immunoreactivity was found. There were 9 tumours with p53 protein accumulation without detectable p53 mutation. Both p53 mutation and p53 immunoreactivity were found in 12 tumours. Mutations of p53 were present in 11 of 26 HPV-positive carcinomas and 3 of 11 HPV-negative tumours. There were no HPV type specific differences between tumours with and without p53 alterations. Only 5 carcinomas had neither HPV DNA nor p53 alterations. Overall we found a slight accumulation of p53 alterations in HPV-positive cases.

Discussion

With adequately designed PCR/Southern blot analysis it has been found by our group and other investigators that oral carcinomas contain high-risk HPV DNA in more than 50% of cases [29, 37, 39]. In our study 3 of 4 carcinomas of the pharynx were also positive for high-risk HPV DNA. This and results of Watts et al. [37] are compatible with the assumption of a similar rate of HPV infections in pharyngeal carcinomas and in oral carcinomas. The role of HPV as an aetiological factor in oropharyngeal carcinogenesis is not as thoroughly investigated as for carcinomas of the cervix uteri. Some results indicate that differences exist between these tumours with respect to their HPV state. The studies of Chang et

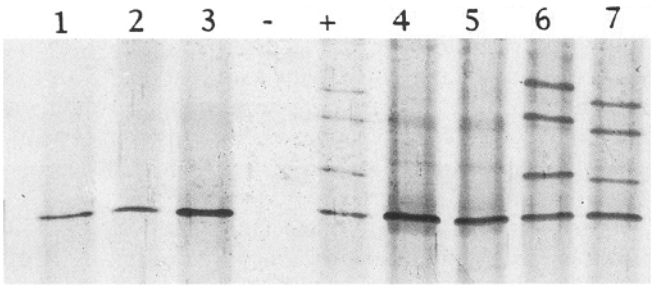

Fig. 4 Polymerase chain reaction (PCR)/temperature gradient gel electrophoresis (TGGE) analysis of exon 7 of p53. Four distinct bands indicate a mutation in two oral carcinomas (*lanes 6 and 7*). + positive control tumor cell line CEM, - negative control (none DNA)

al. [8] and Watts et al. [37] point out that HPV DNA often remains in an episomal state in oral carcinomas, in contrast to the frequent integration events in carcinomas of the cervix uteri. Furthermore our own findings are suggestive of a lower copy number of HPV DNA in oral carcinomas [29]. It is thought that the expression of the HPV oncogenes E6 and E7 is closely related to the tumourigenic potential of HPV 16 and 18. The E6 and E7 oncoproteins inactivate the proteins encoded by the retinoblastoma tumour suppressor gene and the p53 gene [21, 32, 38] and these events probably play a major role in the development of carcinomas of the cervix uteri. Whether this is also true for oropharyngeal carcinomas has not been studied in detail. In view of these data and unresolved problems it seems questionable whether HPV is as operative in oropharyngeal carcinomas as it is in cancer of the cervix uteri.

Some differences between oropharyngeal and cervical squamous cell carcinomas become evident when looking at their epidemiology. Cancer and precancer of the cervix uteri occur mostly in young and middle-aged individuals and are related to sexual behaviour. Oropharyngeal carcinomas arise predominantly in the elderly and are related to alcohol consumption and smoking habits [1, 2, 19, 26]. Critical targets of genomic alterations by chemical carcinogens include tumour suppressor genes and the p53 gene, acting as cell-cycle check point by the induction of growth arrest or apoptosis in DNA damaged cells, is found to be altered at a high rate in common malignancies, such as colorectal cancer and small cell carcinoma of the lung [25]. But there are also tumours with a very low rate of p53 alterations, including squamous cell carcinomas of the cervix uteri, especially HPV-positive cervical carcinomas [10, 11, 16, 22, 24, 30, 33, 38]. Missense p53 mutations convey much more stability to the encoded protein compared with wt p53, resulting in an intensive immunoreactivity in most cases [5, 40], but frame shift mutations or newly inserted stop codons abolish p53 expression and lead to negative immunohistochemical reactions as in most tissues with wt p53 [6]. Although less frequent, p53 immunostaining may also become detectable in the absence of mutations, due to epigenetic influence on protein processing, and it is also affected by tissue fixation, and embedding [14]. These explanations could be the basis of p53 immunoreactivity of nonmutated tumours in our study. In our experience antigen retrieval by microwave heating is a useful step to improve immunocytochemical reactivity and to diminish equivocal results, but in preliminary studies we found no complete p53 nonreactive tumour that became positive after additional antigen retrieval. According to our results the published immunohistochemical data display up to 60% of p53 immunoreactive squamous cell carcinomas in the upper aerodigestive tract [13, 15, 17, 36]. The detection of p53 overexpression in a high rate of betel- and tobacco-related human oral dysplasias and squamous cell carcinomas [23] and the predominant occurrence of p53 accumulation in oral carcinomas of smokers [13] point to the aetiological relevance of these exogenic factors.

For detecting p53 mutations we used the TGGE as screening method. We have previously shown that the TGGE technique has some advantages in comparison to the widely used SSCP, particularly in the visualization of gel shifts and extra bands [34]. Mutant p53 was discovered in the present series of oropharyngeal carcinomas in 14 of 37 tumours (37.8%). This rate is within the range of the small number of other studies on oropharyngeal carcinomas [3–6, 9, 31]. Frequently the G-nucleotide is the site of point mutations, providing further evidence that chemical carcinogens in tobacco smoke play a major role in the aetiology of these tumours [3, 6].

We have shown that p53 mutation and HPV DNA in oropharyngeal squamous cell carcinomas are not inversely correlated as is regularly observed in carcinomas of the cervix uteri. Our results clearly demonstrate that only a small subset of oropharyngeal carcinomas were negative for both HPV DNA and p53 alterations. For the great majority of oropharyngeal carcinomas two alternative or cooperative pathways of p53 alteration may be active. It is tempting to speculate that in HPV-positive tumours with a p53 wild-type state, native suppressor functions are abrogated by interaction with HPV E6 and consecutive degradation of p53-E6 complexes via the ubiquitin pathway [21, 33, 39]. In the heterozygous or homozygous mutant state, the dominant oncogenic effect of mutant p53 may override the p53 inactivation by HPV oncogenes. In accordance with the results of Chang et al. [7] on oesophageal tumours our findings indicate remarkable differences in the concomitant presence of p53 mutations and HPV DNA between squamous cell carcinomas of the genital tract, especially of the cervix uteri, and the upper aerodigestive tract. This could reflect differences in the aetiology of squamous cell carcinomas of different sites. The frequent occurrence of both p53 mutations and HPV DNA in oropharyngeal carcinomas is suggestive of a stronger influence of chemical carcinogens in oncogenesis, in comparison with the probably HPV dominated aetiology of carcinomas of the cervix uteri.

Acknowledgements The authors thank Mrs. Anke Wenzlawski and Mrs. Marianne Thiemann for technical assistance and Mrs. Christa Lukat for preparing the micrographs. This study was supported by the Deutsche Forschungsgemeinschaft.

References

1. Blot WJ, Mc Laughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF Jr (1988) Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 48:3282–3287
2. Bofetta P, Mashberg A, Winkelman R, Garfinkel L (1992) Carcinogenic effect of tobacco smoking and alcohol drinking on anatomic sites of the oral cavity and oropharynx. Int J Cancer 52:530–533
3. Boyle JO, Hakim J, Koch W, van der Riet P, Hruban RH, Roa RA, Correo R, Eby YJ, Sidransky D (1993) The incidence of p53 mutations increases with progression of head and neck cancer. Cancer Res 53:4477–4480
4. Brachman DG, Graves D, Vokes E, Beckett M, Haraf D, Montag A, Dunphy E, Mick R, Yandell D, Weichselbaum RR (1992) Occurrence of p53 gene deletions and human papilloma virus infection in human head and neck cancer. Cancer Res 52:4832–4836
5. Burns JE, Baird MC, Clark LJ, Burns PA, Edington K, Mitchell R, Robertson G, Soutar D, Parkinson EK (1993) Gene mutations and increased levels of p53 protein in human squamous cell carcinomas and their cell lines. Br J Cancer 67:1274–1284
6. Caamano J, Zhang SY, Rosvold EA, Bauer B, Klein-Szanto AJP (1993) p53 alterations in human squamous cell carcinomas and carcinoma cell lines. Am J Pathol 142:1131–1139
7. Chang F, Syrjänen S, Tervahauta A, Kurvinen K, Wang L, Syrjänen K (1994) Frequent mutations of p53 gene in oesophageal squamous cell carcinomas with and without human papillomavirus (HPV) involvement suggest the dominant role of environmental carcinogens in oesophageal carcinogenesis. Br J Cancer 70:346–351
8. Chang KW, Chang CS, Lai KS, Chou MJ, Choo KB (1989) High prevalence of human papillomavirus infection and possible association with betel quid chewing and smoking in oral epidermoid carcinomas in Taiwan. J Med Virol 28:57–61
9. Chang YS, Lin YJ, Shu CH, Tsai MSh, Choo KB, Liu ST (1992) Detection of mutations in the p53 gene in human head and neck carcinomas by single strand conformation polymorphism analysis. Cancer Lett 67:167–174
10. Crook T, Wrede D, Vousden KH (1991) p53 point mutation in HPV negative human cervical carcinoma cell lines. Oncogene 6:873–875
11. Crook T, Wrede D, Tidy JA, Mason WP, Evans DJ, Vousden KH (1992) Clonal p53 mutation in primary cervical cancer: association with human papillomavirus-negative tumours. Lancet 339:1070–1073
12. Dallas P, Flanagan J, Nightingale B, Morris B (1989) Polymerase chain reaction for fast, nonradioactive detection of high- and low-risk papillomavirus types in routine cervical specimens and in biopsies. J Med Virol 27:105–111
13. Field JK, Spandidos DA, Malliri A, Gosney JR, Yiagnisis M, Stell PM (1991) Elevated p53 expression correlates with a history of heavy smoking in squamous cell carcinomas of the head and neck. Br J Cancer 64:573–577
14. Fisher CJ, Gillet CE, Vojesek B, Barnes DM, Millis RR (1994) Problems with p53 immunohistochemical staining: the effect of fixation and variation in the methods of evaluation. Br J Cancer 69:26–31
15. Frank JL, Bur ME, Garb JL, Kay S, Ware JL, Sismanis A, Neifeld JP (1994) p53 tumour suppressor oncogene expression in squamous cell carcinoma of the hypopharynx. Cancer 73:181–186
16. Fujita M, Inoue M, Tanizawa O, Iwamoto S, Enomoto T (1992) Alterations of the p53 gene in human primary cervical carcinoma with and without human papillomavirus infection. Cancer Res 52:5323–328
17. Gusterson BA, Anbazhagan R, Warren W, Migdely C, Lane DP, O'Hare M, Stamps A, Carter R, Jayatilake H (1991) Expression of p53 in premalignant and malignant squamous epithelium. Oncogene 6:1785–1789
18. Hickman ES, Picksley SM, Vousden KH (1994) Cells expressing HPV 16 E7 continue cell cycle progression following DNA damage induced p53 activation. Oncogene 9:2177–2181
19. Hirayama T (1966) An epidemiological study of oral and pharyngeal cancer in Central and South-East Asia. Bull World Health Organ 34:41–69
20. Hollstein M, Sidransky Y, Vogelstein B, Harris CC (1991) p53 mutations in human cancers. Science 253:49–53
21. Hoppe-Seyler F, Butz K (1993) Repression of endogenous p53 transactivation function in HeLa cervical carcinoma cells by human papillomavirus type 16 E6, human mdm-2, and mutant p53. J Virol 67:3111–3117
22. Kaelbling M, Burk RD, Atkin NB, Johnson AB, Klinger HP (1992) Loss of heterozygosity on chromosome 17p and mutant p53 in HPV-negative cervical carcinomas. Lancet 340:140–42
23. Kaur J, Srivastava A, Ralhan R (1994) Overexpression of p53 protein in betel- and tobacco-related human oral dysplasia and squamous-cell carcinoma in India. Int J Cancer 58:340–345
24. Kessis TD, Slebos RJ, Han SM, Shah KF, Bosch XF, Munoz N, Hedick L, Cho KR (1993) p53 gene mutations and MDM2 amplification are uncommon in primary carcinomas of the uterine cervix. Am J Pathol 143:1398–1405
25. Levine J (1992) The p53 tumour-suppressor gene. N Engl J Med 326:1350–1352
26. Merletti F, Bofetta P, Cicone G, Mashberg A, Terracini B (1989) Role of tobacco and alcoholic beverages in the etiology of cancer of the oral cavity/oropharynx in Torino/Italy. Cancer Res 49:4919–4924
27. Milde K, Löning T (1986) Detection of papillomavirus DNA in oral papillomas and carcinomas: application of in situ hybridization with biotinylated HPV 16 probes. J Oral Pathol Med 15:296–306
28. Ogden GR, Kiddie RA, Lunny DP, Lane DP (1992) Assessment of p53 protein expression in normal, benign and malignant oral mucosa. J Pathol 166: 389–394
29. Ostwald C, Müller P, Barten M, Rutsatz K, Sonnenburg M, Milde-Langosch K, Löning T (1994) Human papillomavirus DNA in oral squamous cell carcinomas and normal mucosa. J Oral Pathol Med 23:220–225
30. Paquette RL, Wilczynski SP, Karmakar A, Kizaki M, Miller CW, Koeffler HP (1993) Mutations of p53 and human papillomavirus infection in cervical carcinoma. Cancer 72:1272–1280
31. Sakai E, Tsuchida N (1992) Most human squamous cell carcinoma in the oral cavity contain mutated p53 tumour-suppressor genes. Oncogene 7:927–933
32. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM (1990) The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes degradation of p53. Cell 63:1129–1136
33. Scheffner M, Münger K, Byrne JC, Howley PM (1991) The state of p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc Natl Acad Sci USA 88:5523–5527
34. Scholz RB, Milde-Langosch K, Jung R, Schlechte H, Kabisch H, Wagener RC, Löning T (1993) Rapid screening for Tp53 mutations by temperature gradient gel electrophoresis: a comparison with SSCP analysis. Hum Mol Genet 2:2155–2158
35. Shibata DK, Arnheim J, Martin WJ (1988) Detection of human papillomavirus in paraffin-embedded tissue using the polymerase chain reaction. J Exp Med 167:225–230
36. Warnakulasuriya KAAS, Johnson NW (1992) Expression of p53 mutant nuclear phosphoprotein in oral carcinoma and potentially malignant oral lesions. J Oral Pathol Med 21:404–408
37. Watts SL, Brewer E, Fry T (1991) Human papillomavirus DNA types in squamous cell carcinomas of the head and neck. Oral Surg Oral Med Oral Pathol 71:701–707
38. Werness BA, Levine AJ, Howley PM (1990) Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 248:76–79
39. Woods KV, Shillitoe EJ, Spitz MR, Schantz SP, Adler-Stohrtz K (1993) Analysis of human papillomavirus DNA in oral squamous cell carcinomas. J Oral Pathol Med 22:101–108
40. Wynford-Thomas (1992) p53 tumour pathology: can we trust immunocytochemistry? J Pathol 166: 329–330
41. Zur Hausen H (1994) Molecular pathogenesis of cancer of the cervix and its causation by specific HPV types. Curr Top Microbiol Immunol 186:131–156