

p53 REVIEW ARTICLE

TP53 and Head and Neck Neoplasms

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For the p53 Special Issue

Head and neck cancer is an important health problem around the world, accounting for approximately 500,000 new cases each year of head and neck squamous cell carcinoma (HNSCC). Carcinogenesis of head and neck results from a dysregulation of cellular proliferation, differentiation, and cell death. The major etiologic agents are tobacco and alcohol consumption and for some cases, human papilloma virus (HPV) infection. All three factors are associated with the disruption of a cellular pathway essential for the maintenance of cellular integrity, the p53 pathway. The objective of this review is to point out the specificity of p53 gene (TP53) alterations in head and neck cancer in relation with chemocarcinogenesis and to discuss whether or not the determination of p53 alterations will be of clinical relevance in the management of head and neck cancer in terms of prognosis and response to treatments. *Hum Mutat* 21:252–257, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: p53; TP53; cancer; tumor; neoplasm; head; neck; squamous cell carcinoma; tobacco; alcohol; carcinogen

DATABASES:

TP53 – OMIM: 191170; GenBank: NM_000546 (mRNA)

<http://p53.curie.fr/> (p53 Web Site at Institut Curie)www.iarc.fr/p53 (IARC p53 Mutation Database)

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) accounts for 5% of all newly diagnosed cancer cases in northern and western European countries and in the United States [Muir and Weiland, 1995]. It is one of the most common cancers, with a worldwide global incidence of 500,000 cases per year [Parkin et al., 1988]. The etiology of HNSCC has been clearly defined in terms of risk factors and the role of tobacco and alcohol consumption is well established [Tuyns et al., 1988]. Direct effects of tobacco on the occurrence of head and neck cancer is reinforced by the fact that different tobacco habits can influence tumor localization, as shown by the high incidence of oral cancers in tobacco chewers and by the correlation observed between the level of tobacco or alcohol exposure and the prevalence of the disease [Ahrendt et al., 2000; Balaram et al., 2002]. Other risk factors include environmental exposures to wool dust, wood dust, mineral fibers, low intake of vegetables and fruits, and human papilloma-virus infection (HPV16) [Mork et al., 2001].

One of the most frequent alterations in HNSCC is the disruption of the p53 pathway (TP53; MIM# 191170) through mutations, losses of heterozygosity,

or interaction with viral proteins. Alterations in p53 are frequent, appear at early stages of head and neck carcinogenesis, and are of particular relevance, since both tobacco carcinogens and HPV infection, two major risk factors, appear to target p53. Moreover, head and neck cancer is, along with lung cancer [Toyooka et al., 2003], a tumor site for which p53 gene therapy has been developed, demonstrating various degrees of success [Nemunaitis et al., 2001]. Many studies have dealt with p53 in HNSCC and numerous technologies have been described for the identification of p53 alterations including immunohistochemistry, mutation screenings, antibody detection, and functional tests in yeast. In the following review, we did not consider studies using immunohistochemical detection of p53 as a marker for gene mutation since this method has lead to over 40%

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discordance with sequencing [Ma et al., 1998; Taylor et al., 1999].

PREVALENCE AND ETIOLOGY OF TP53 MUTATION IN HNSCC

The prevalence of p53 mutations in HNSCC varies from 30 to 70% among studies of the literature [Alsner et al., 2001; Brennan et al., 1995a; Cabelguenne et al., 2000; Gillison et al., 2000; Ko et al., 2001; Temam et al., 2000]. The heterogeneity of the different studies in terms of tumor localization and mutation detection technologies could partially explain the variation observed in the incidence of p53 mutations. However, the main reason could be different levels of exposure to risk factors in the populations studied.

The frequency of p53 gene mutations in patients with invasive HNSCC was related to the level of exposure to cigarette smoke and alcohol. The frequency of somatic mutations in the p53 gene in smokers was twice as high as that of non-smokers [Ko et al., 2001]. Our personal data confirmed these results, showing a dose-dependant effect of tobacco on the frequency of p53 mutations. In a series of 140 patients the frequency of p53 mutations was 74%, 66%, and 35.5% in patients who had smoked more than 35 pack-years, 15 to 35 pack-years, and less than 15 pack years respectively. Furthermore, the frequency of somatic mutations in the p53 gene was 3.5 times more common among patients who both smoked cigarettes and drank alcohol than in patients who neither smoked nor drank [Brennan et al., 1995a].

The specific role of alcohol in the occurrence of p53 mutations is hard to prove, since alcohol abuse is frequently associated with a tobacco habit. In only one series the specific positive impact of alcohol on the incidence of p53 mutations was significant after adjustment for cigarette smoking and betel quid in a Taiwanese series of patients [Hsieh et al., 2001]. On the contrary, HPV-positive patients were less likely to harbor p53 mutated tumors. In a subgroup of oropharyngeal tumors, TP53 was inversely associated with HPV; 67% of HPV-negative but only 10% of HPV-positive tumors were p53 mutated [Gillison et al., 2000]. Despite the importance of lifestyle, host susceptibility could also influence the occurrence of p53 alteration in HNSCC.

DNA can be damaged by numerous tobacco carcinogenes and environmental chemicals that can be activated or degraded by specific enzymes termed xenobiotic-metabolizing enzymes (XMEs). The existence of XME variants could explain individual susceptibility to p53 mutations. An XME genotype that results in increased DNA damaged as a consequence of altered carcinogen metabolism could increase the incidence of mutations in the p53 gene. Bio-activation of pro-carcinogenes into an active state

is catalyzed by phase I enzyme as cytochrome P450 mono-oxygenase. A strong association between aberration frequencies in the p53 gene and CYP1B1 genotypes was found in smokers. Patients with the 1*/2* or 2*/2* genotypes were (seven and 20 times, respectively) more likely to show smoking-induced somatic mutations in the p53 gene than those with CYP1B1 wild-type genotype. This indicates that the exchange of amino acid valine to leucine at codon 432 (Val432Leu) is strongly associated with the frequency of smoking-induced p53 gene mutations [Ko et al., 2001; Thier et al., 2002].

Bio-inactivation of chemicals with carcinogenic potential is mostly done by phase II enzymes as the GST superfamily genes. Concerning phase II enzyme, a correlation between p53 mutations and GSTP1 homozygous genotype VAL105 was observed in our series. p53 mutations were found more frequently in individuals with homozygous GSTP1 val 105 genotype (93%) compared with GSTP1 val105/GSTP1ile 105 (69%) or homozygous GSTP1 ile (61%) [Cabelguenne et al., 2001]. Finally, increased sensitivity to mutagenes as a result of low DNA repair capacities could increase the frequency or modify the pattern of p53 mutations. The XRCC1 Q allele (Q399R) was related with, respectively, the incidence of p53 mutations and the existence of G to A transitions in two studies [Casse et al., 2002; Wong et al., 2002].

PATTERNS OF TP53 MUTATIONS IN HEAD AND NECK CANCER

The pattern of p53 point mutations encountered in HNSCC is given in Figure 1. As expected from experimental tobacco carcinogenesis, the most prevalent mutations are G:C>A:T transitions and G:C>T:A transversions. It is interesting to note that frameshift mutations (i.e., deletions and insertions) occur significantly more frequently in HNSCC than in other cancers. They occur approximately in 16% of cases, whereas this type of mutations occurs in less than 9% of all other cancers in the p53 IARC database [Olivier et al., 2002]. It was noticed by Brennan et al. [1995a] that this type of mutation occurs more frequently in the sub-group of patients both exposed to alcohol and tobacco [Tabor et al., 2001]. To confirm the fact that the association of both risk factors leads to enhanced frequency of deletion and insertion (frame-shift mutations), we compared, in the p53 IARC database, the prevalence of these alterations in two other cancer types. The prevalence of frame-shift mutations was 17.3% in a series of 860 p53 mutations detected in squamous cell carcinoma of the esophagus, a strongly tobacco- and alcohol-related cancer, and only 11.5% in a series of 544 squamous cell carcinoma of the lung, a cancer linked only to tobacco abuse. This suggests that indeed, tobacco and alcohol trigger frame-shift mutations in p53.

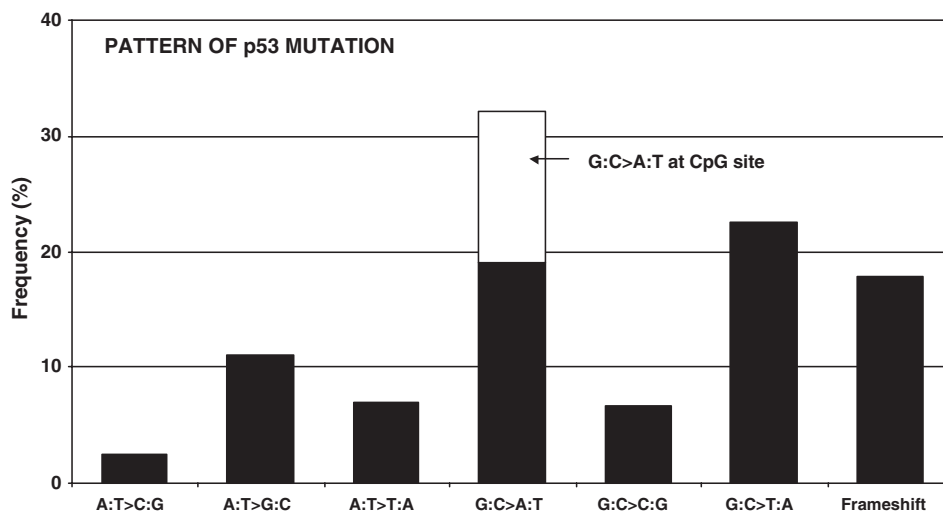


FIGURE 1. The pattern of p53 point mutations encountered in HNSCC. Upper portion of center bar represents frequency of G:C>A:T transitions associated with CpG dinucleotides. Data were derived from the IARC p53 Mutation Database (www.iarc.fr/p53). For a discussion of p53 mutations at CpG dinucleotides in human cancers, see Soussi and Beroud [2003].

TP53 MUTATIONS OCCUR IN EARLY STEP OF HEAD AND NECK CARCINOGENESIS

Multiple genetic alterations are seen in HNSCC and it is now evident that these cancers arise from multistep carcinogenesis. p53 changes occur early in head and neck carcinogenesis and can be detected in premalignant lesions [Boyle et al., 1993]. Moreover, direct sequencing of full-length p53 showed no difference in terms of p53 mutations when comparing primary tumor and match nodal metastasis. This suggested that p53 mutations are present before and maintained in the metastasis [Tjebbes et al., 1999]. The high risk of second primary tumor in the upper digestive tract is one of the clinical characteristics of HNSCC leading to a constant rate of 2–3% new cases per year. The analysis of DNA extracted from normal epithelium at a distance of over 0.5 cm from the tumor margins for p53 mutations showed in 3 out of 8 samples identical p53 mutation as compared with primary tumor [Tabor et al., 2001].

Although this result strongly indicates a clonal relationship between the surrounding fields and the tumor, it is interesting to note that there were other genetic differences between fields and tumor as shown by microsatellite instability. This result is in favor of an early occurrence of p53 mutations in the clonal cancerization field process. Finally, p53 mutations have been observed at all steps of HNSCC carcinogenesis but no mutations have ever been found in normal mucosa never exposed to carcinogen [Lavielle et al., 1998]. Although there is an increasing prevalence of p53 protein expression with increasingly severe grades of dysplasia [Califano et al., 1996], the usefulness of this molecular marker to predict malignant potential of oral cancer has not yet been demonstrated [Warnakulasuriya, 2000].

CLINICO-PATHOLOGICAL IMPLICATION OF P53 MUTATIONS IN HNSCC

Residual Disease and Follow-Up

Molecular probing using p53 has been tested in different trials and samples. Huang et al. [1999] showed that p53 alterations could be found in exfoliative cells from oral mucosa in head and neck cancer patients. Moreover, mucosal p53 overexpression has been associated with an increased incidence of second primary cancer [Homann et al., 2001; Nathan et al., 2000]. These studies suggested that the identification of p53 alterations could be of use in follow-up HNSCC patients. Additional works showed that p53 mutations could be detected in histologically normal tissues at the margins of resected HNSCC. This could also have clinical implications in terms of molecular assessment of histopathological staging in squamous cell carcinoma [Brennan et al., 1995b]. Indeed, one of the most important prognostic factors in HNSCC is complete surgical resection of the tumor. The presence of microscopic residual tumor cell in tumor margin increases the rate of local recurrence [Brennan et al., 1991; Davidson et al., 1981]. A series of patients with p53 mutated tumors and complete resection on the basis of negative histology of tumor margins was selected by Brennan et al. [1995b]. After identification of p53 mutations in tumors they looked for similar alterations in surgical margins. Thirteen patients were found positive for p53 mutations in margins. Among them 38% have recurred locally as compared with none of the 12 patients with negative margins [Brennan et al., 1995b]. p53 mutations were also retrieved in lymph node, saliva, or serum samples [Boyle et al., 1994; Coulet et al., 2000; Tjebbes et al., 1999]. In all cases the main goal was the identification of rare tumor cells by p53 mutation-based assays. This

technology has two major limits, first it concerns only an average percentage of patients of 60% for which a mutated p53 tumor was diagnosed and second, the heterogeneity of p53 mutations is a barrier to establishing simple mutation detection assays. [for review see van Houten et al., 2000].

Prognostic Significance of p53 Mutation in Head and Neck Carcinoma

Correlation between the occurrence of p53 alteration and poor prognosis in HNSCC patients is still a matter of debate. Indeed, overexpression of p53 has been related to a worse prognosis in HNSCC [Jin et al., 1998; Shin et al., 2000] although this result is still controversial and many studies did not find any relation [Georgiou et al., 2001; Gonzalez-Moles et al., 2001; Hirvikoski et al., 1997; Jeannon et al., 2000; Kokoska et al., 1996; Sittel et al., 2000]. The prognostic value of p53 mutations is far less controversial as several studies reported a worse prognosis for patients with p53 mutated tumors [Bandoh et al., 2002; Bradford et al., 1997; Mineta et al., 1998]. Finally, the accumulation of mutant p53 protein leads to the production of anti-p53 antibodies which has been found up to 17–19% in the largest series published [Bourhis et al., 1996; Cabelguenne et al., 2000]. The presence of anti-p53 antibodies is significantly associated with increased risks of relapse and death. The overall survival proportion at 2 years was 63% when no anti-p53 antibodies were detected as compared to 29% when anti-p53 antibodies were detected. Furthermore in one series it has been shown that anti-p53 antibodies were associated with the occurrence of lymph nodes metastasis [Chow et al., 2001].

Differences observed between series in terms of impact of p53 alterations on prognosis could be due to different modalities of treatment. Indeed a relation seems to exist between p53 alterations and response to treatment. The presence of p53 mutations was associated with overexpression of bcl-2, locoregional failure, and worse survival in a patient treated with radiotherapy suggesting that these genes are an important determinant of radiation-induced apoptosis in HNSCC [Gallo et al., 1999]. This result has been confirmed by others who also showed that this relation could not be found for patients treated by surgery [Alsner et al., 2001]. The role of chemotherapy in HNSCC is matter of debate in the multimodality therapy of locoregionally advanced tumors and the impact of p53 mutations in response to chemotherapy appears to be of clinical relevance in HNSCC patients. Two independent series showed similar results indicating that p53 mutations could, independently from other clinical parameters, predict the absence of objective response in head and neck cancer patients treated by 5 fluorouracil-cisplatin

based neoadjuvant chemotherapy. Furthermore in the case of p53 mutations located in the DNA interacting domain (i.e., L3 loop, H2 loop sheet helix, S10 β strand, zinc binding residues) the chance of major response to chemotherapy is even minored [Cabelguenne et al., 2000; Temam et al., 2000].

TP53 as Gene Therapy

HNSCC has been one of the first tumor locations to benefit from gene transfer therapy. It was shown that transfection of wild-type p53 into cell lines induced growth arrest and reduced tumorigenicity in nude mice [Liu et al., 1994]. This suggested that restoring p53 function in head and neck tumors could trigger cell growth. Therapeutic strategies based on p53 tumor suppressor functions were initiated. Phase I and II were completed with adenovirus-mediated wild-type p53 gene transfer as a surgical adjuvant in advanced head and neck cancers [Clayman et al., 1998; Clayman et al., 1999]. Although this work was preliminary, results in terms of security and tumor responses suggested that adenovirus-mediated wild-type p53 gene transfer could be used as a surgical adjuvant in patients with advanced HNSCC.

CONCLUSIONS

The p53 protein is involved in the maintenance of the cellular integrity after DNA damage. In a cancer type for which chemocarcinogenesis is of major importance, the disruption of the p53 pathway could lead to intense genetic or genomic instability and trigger carcinogenesis. Indeed, alterations in p53, through losses of heterozygosity, point mutations, deletions, insertions, or interaction with viral proteins are common and mark early events in head and neck carcinogenesis.

The etiology of most p53 alterations has been clearly related to the exposure of epithelium to tobacco carcinogens and to alcohol intake, which demonstrated that p53 alterations are indeed at the center of head and neck chemocarcinogenesis. Moreover, p53 alterations have been related to various anatomoclinic parameters, suggesting that p53 could be a useful molecular marker in HNSCC.

The clinical impact of detecting p53 alterations in tumor surgical margins, lymph nodes, or body fluids such as saliva or blood to detect residual disease still needs to be evaluated in larger studies. However it seems promising, and the predictive role of p53 mutations on treatment responses, in particular in the case of radiotherapy or chemotherapy, was demonstrated. Finally the evaluation of p53 gene transfer as adjuvant treatment in advanced head and neck cancer has led to some degree of success and needs to be confirmed or evaluated in a multimodal therapy.

REFERENCES

- Ahrendt SA, Chow JT, Yang SC, Wu L, Zhang MJ, Jen J, Sidransky D. 2000. Alcohol consumption and cigarette smoking increase the frequency of p53 mutations in non-small cell lung cancer. *Cancer Res* 60:3155–3159.
- Alsner J, Sorensen SB, Overgaard J. 2001. TP53 mutation is related to poor prognosis after radiotherapy, but not surgery, in squamous cell carcinoma of the head and neck. *Radiother Oncol* 59:179–185.
- Balaram P, Sridhar H, Rajkumar T, Vaccarella S, Herrero R, Nandakumar A, Ravichandran K, Ramdas K, Sankaranarayanan R, Gajalakshmi V, Munoz N, Franceschi S. 2002. Oral cancer in southern India: the influence of smoking, drinking, paan-chewing and oral hygiene. *Int J Cancer* 98:440–445.
- Bandoh N, Hayashi T, Kishibe K, Takahara M, Imada M, Nonaka S, Harabuchi Y. 2002. Prognostic value of p53 mutations, bax, and spontaneous apoptosis in maxillary sinus squamous cell carcinoma. *Cancer* 94:1968–1980.
- Bourhis J, Lubin R, Roche B, Koscielny S, Bosq J, Dubois I, Talbot M, Marandas P, Schwaab G, Wibault P, Luboiniski B, Eschwege F, Soussi T. 1996. Analysis of p53 serum antibodies in patients with head and neck squamous cell carcinoma. *J Natl Cancer Inst* 88:1228–1233.
- Boyle JO, Hakim J, Koch W, van der Riet P, Hruban RH, Roa RA, Correo R, Eby YJ, Ruppert JM, Sidransky D. 1993. The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res* 53:4477–4480.
- Boyle JO, Mao L, Brennan JA, Koch WM, Eisele DW, Saunders JR, Sidransky D. 1994. Gene mutations in saliva as molecular markers for head and neck squamous cell carcinomas. *Am J Surg* 168:429–432.
- Bradford CR, Zhu S, Poore J, Fisher SG, Beals TF, Thoraval D, Hanash SM, Carey TE, Wolf GT. 1997. p53 mutation as a prognostic marker in advanced laryngeal carcinoma. Department of veterans affairs laryngeal cancer cooperative study group. *Arch Otolaryngol Head Neck Surg* 123:605–609.
- Brennan CT, Sessions DG, Spitznagel EL Jr, Harvey JE. 1991. Surgical pathology of cancer of the oral cavity and oropharynx. *Laryngoscope* 101:1175–1197.
- Brennan JA, Boyle JO, Koch WM, Goodman SN, Hruban RH, Eby YJ, Couch MJ, Forastiere AA, Sidransky D. 1995a. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. *N Engl J Med* 332:712–717.
- Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, Goodman SN, Sidransky D. 1995b. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N Engl J Med* 332:429–435.
- Cabelguenne A, Blons H, de Waziers I, Carnot F, Houllier AM, Soussi T, Brasnu D, Beaune P, Laccourreye O, Laurent-Puig P. 2000. p53 alterations predict tumor response to neoadjuvant chemotherapy in head and neck squamous cell carcinoma: a prospective series. *J Clin Oncol* 18:1465–1473.
- Cabelguenne A, Loriot MA, Stucker I, Blons H, Koum-Besson E, Brasnu D, Beaune P, Laccourreye O, Laurent-Puig P, De Waziers I. 2001. Glutathione-associated enzymes in head and neck squamous cell carcinoma and response to cisplatin-based neoadjuvant chemotherapy. *Int J Cancer* 93:725–730.
- Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, Corio R, Lee D, Greenberg B, Koch W, Sidransky D. 1996. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 56:2488–2492.
- Casse C, Chuan Hu Y, Benoit N, Sidransky D, Ahrendt SA. 2002. The XRCC1 codon 399 polymorphism is associated with G to A transitions in p53 non-small cell lung cancer. *Proc AACR 2002 abstract* 2003. p 3210.
- Chow V, Yuen AP, Lam KY, Ho WK, Wei WI. 2001. Prognostic significance of serum p53 protein and p53 antibody in patients with surgical treatment for head and neck squamous cell carcinoma. *Head Neck* 23:286–291.
- Clayman GL, el-Naggar AK, Lippman SM, Henderson YC, Frederick M, Merritt JA, Zumstein LA, Timmons TM, Liu TJ, Ginsberg L, Roth JA, Hong WK, Bruso P, Goepfert H. 1998. Adenovirus-mediated p53 gene transfer in patients with advanced recurrent head and neck squamous cell carcinoma. *J Clin Oncol* 16:2221–2232.
- Clayman GL, Frank DK, Bruso PA, Goepfert H. 1999. Adenovirus-mediated wild-type p53 gene transfer as a surgical adjuvant in advanced head and neck cancers. *Clin Cancer Res* 5:1715–1722.
- Coulet F, Blons H, Cabelguenne A, Lecomte T, Lacourreye O, Brasnu D, Beaune P, Zucman J, Laurent-Puig P. 2000. Detection of plasma tumor DNA in head and neck squamous cell carcinoma by microsatellite typing and p53 mutation analysis. *Cancer Res* 60:707–711.
- Davidson TM, Nahum AM, Astarita RW. 1981. Microscopic controlled excisions for epidermoid carcinoma of the head and neck. *Otolaryngol Head Neck Surg* 89:244–251.
- Gallo O, Chiarelli I, Boddi V, Bocciolini C, Bruschini L, Porfirio B. 1999. Cumulative prognostic value of p53 mutations and bcl-2 protein expression in head-and-neck cancer treated by radiotherapy. *Int J Cancer* 84:573–579.
- Georgiou A, Gomatos IP, Ferekidis E, Syrigos K, Bistola V, Giotakis J, Adamopoulos G, Androulakis G. 2001. Prognostic significance of p53, bax and bcl-2 gene expression in patients with laryngeal carcinoma. *Eur J Surg Oncol* 27:574–580.
- Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. 2000. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 92:709–720.
- Gonzalez-Moles MA, Galindo P, Gutierrez-Fernandez J, Sanchez-Fernandez E, Rodriguez-Archilla A, Ruiz-Avila I, Bravo M. 2001. P53 protein expression in oral squamous cell carcinoma. survival analysis. *Anticancer Res* 21:2889–2894.
- Hirvikoski P, Kumpulainen E, Virtaniemi J, Johansson R, Haapasalo H, Marin S, Halonen P, Helin H, Raitiola H, Pukander J, Kellokumpu-Lehtinen P, Kosma VM. 1997. p53 expression and cell proliferation as prognostic factors in laryngeal squamous cell carcinoma. *J Clin Oncol* 15:3111–3120.
- Homann N, Nees M, Conradt C, Dietz A, Weidauer H, Maier H, Bosch FX. 2001. Overexpression of p53 in tumor-distant epithelia of head and neck cancer patients is associated with an increased incidence of second primary carcinoma. *Clin Cancer Res* 7:290–296.
- Hsieh LL, Wang PF, Chen IH, Liao CT, Wang HM, Chen MC, Chang JT, Cheng AJ. 2001. Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. *Carcinogenesis* 22:1497–1503.

- Huang MF, Chang YC, Liao PS, Huang TH, Tsay CH, Chou MY. 1999. Loss of heterozygosity of p53 gene of oral cancer detected by exfoliative cytology. *Oral Oncol* 35:296–301.
- Jeannon JP, Soames J, Lunec J, Awwad S, Ashton V, Wilson JA. 2000. Expression of cyclin-dependent kinase inhibitor p21(WAF1) and p53 tumour suppressor gene in laryngeal cancer. *Clin Otolaryngol* 25:23–27.
- Jin YT, Kayser S, Kemp BL, Ordonez NG, Tucker SL, Clayman GL, Goepfert H, Luna MA, Batsakis JG, El-Naggar AK. 1998. The prognostic significance of the biomarkers p21WAF1/CIP1, p53, and bcl-2 in laryngeal squamous cell carcinoma. *Cancer* 82:2159–2165.
- Ko Y, Abel J, Harth V, Brode P, Antony C, Donat S, Fischer HP, Ortiz-Pallardo ME, Thier R, Sachinidis A, Vetter H, Bolt HM, Herberhold C, Bruning T. 2001. Association of CYP1B1 codon 432 mutant allele in head and neck squamous cell cancer is reflected by somatic mutations of p53 in tumor tissue. *Cancer Res* 61:4398–4404.
- Kokoska MS, Piccirillo JF, el-Mofty SK, Emami B, Haughey BH, Schoinick SB. 1996. Prognostic significance of clinical factors and p53 expression in patients with glottic carcinoma treated with radiation therapy. *Cancer* 78:1693–1700.
- Lavieille JP, Gazzeri S, Riva C, Reyt E, Brambilla C, Brambilla E. 1998. p53 mutations and p53, Waf-1, Bax and Bcl-2 expression in field cancerization of the head and neck. *Anticancer Res* 18:4741–4749.
- Liu TJ, Zhang WW, Taylor DL, Roth JA, Goepfert H, Clayman GL. 1994. Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant adenovirus. *Cancer Res* 54:3662–3667.
- Ma L, Ronai A, Riede UN, Kohler G. 1998. Clinical implication of screening p53 gene mutations in head and neck squamous cell carcinomas. *J Cancer Res Clin Oncol* 124:389–396.
- Mineta H, Borg A, Dictor M, Wahlberg P, Akervall J, Wennerberg J. 1998. p53 mutation, but not p53 overexpression, correlates with survival in head and neck squamous cell carcinoma. *Br J Cancer* 78:1084–1090.
- Mork J, Lie AK, Glatre E, Hallmans G, Jellum E, Koskela P, Moller B, Pukkala E, Schiller JT, Youngman L, Lehtinen M, Dillner J. 2001. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 344:1125–1131.
- Muir C, Weiland L. 1995. Upper aerodigestive tract cancers. *Cancer* 75:147–153.
- Nathan CA, Sanders K, Abreo FW, Nassar R, Glass J. 2000. Correlation of p53 and the proto-oncogene eIF4E in larynx cancers: prognostic implications. *Cancer Res* 60:3599–3604.
- Nemunaitis J, Khuri F, Ganly I, Arseneau J, Posner M, Vokes E, Kuhn J, McCarty T, Landers S, Blackburn A, Romel L, Randlev B, Kaye S, Kirn D. 2001. Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J Clin Oncol* 19:289–298.
- Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. 2002. The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum Mutat* 19:607–614.
- Parkin DM, Laara E, Muir CS. 1988. Estimates of the worldwide frequency of sixteen major cancers in 1980. *Int J Cancer* 41:184–197.
- Shin DM, Mao L, Papadimitrakopoulou VM, Clayman G, El-Naggar A, Shin HJ, Lee JJ, Lee JS, Gillenwater A, Myers J, Lippman SM, Hittelman WN, Hong WK. 2000. Biochemopreventive therapy for patients with premalignant lesions of the head and neck and p53 gene expression. *J Natl Cancer Inst* 92:69–73.
- Sittel C, Eckel HE, Damm M, von Pritzbuer E, Kvasnicka HM. 2000. Ki-67 (MIB1), p53, and Lewis-X (LeuM1) as prognostic factors of recurrence in T1 and T2 laryngeal carcinoma. *Laryngoscope* 110:1012–1017.
- Soussi T, Beroud C. 2003. Significance of p53 mutations in human cancer: a critical analysis of mutations at CpG dinucleotides. *Hum Mutat* 21:192–200.
- Tabor MP, Brakenhoff RH, van Houten VM, Kummer JA, Snel MH, Snijders PJ, Snow GB, Leemans CR, Braakhuis BJ. 2001. Persistence of genetically altered fields in head and neck cancer patients: biological and clinical implications. *Clin Cancer Res* 7:1523–1532.
- Taylor D, Koch WM, Zahurak M, Shah K, Sidransky D, Westra WH. 1999. Immunohistochemical detection of p53 protein accumulation in head and neck cancer: correlation with p53 gene alterations. *Hum Pathol* 30:1221–1225.
- Temam S, Flahault A, Perie S, Monceaux G, Coulet F, Callard P, Bernaudin JF, St Guily JL, Fouret P. 2000. p53 gene status as a predictor of tumor response to induction chemotherapy of patients with locoregionally advanced squamous cell carcinomas of the head and neck. *J Clin Oncol* 18:385–394.
- Thier R, Bruning T, Roos PH, Bolt HM. 2002. Cytochrome P450 1B1, a new keystone in gene-environment interactions related to human head and neck cancer? *Arch Toxicol* 76:249–256.
- Tjebbes GW, Leppers vd Straat FG, Tilanus MG, Hordijk GJ, Slootweg PJ. 1999. p53 tumor suppressor gene as a clonal marker in head and neck squamous cell carcinoma: p53 mutations in primary tumor and matched lymph node metastases. *Oral Oncol* 35:384–389.
- Toyooka S, Tsuda T, Gazdar AF. 2003. The TP53 gene, tobacco exposure and lung cancer. *Hum Mutat* 21:229–239.
- Tuyns AJ, Esteve J, Raymond L, Berrino F, Benhamou E, Blanchet F, Boffetta P, Crosignani P, del Moral A, Lehmann W, et al. 1988. Cancer of the larynx/hypopharynx, tobacco and alcohol: IARC international case-control study in Turin and Varese (Italy), Zaragoza and Navarra (Spain), Geneva (Switzerland) and Calvados (France). *Int J Cancer* 41:483–491.
- van Houten VM, Tabor MP, van den Brekel MW, Denkers F, Wishaupt RG, Kummer JA, Snow GB, Brakenhoff RH. 2000. Molecular assays for the diagnosis of minimal residual head-and-neck cancer: methods, reliability, pitfalls, and solutions. *Clin Cancer Res* 6:3803–3816.
- Warnakulasuriya S. 2000. Lack of molecular markers to predict malignant potential of oral precancer. *J Pathol* 190:407–409.
- Wong RH, Du CL, Wang JD, Chan CC, Luo JC, Cheng TJ. 2002. XRCC1 and CYP2E1 polymorphisms as susceptibility factors of plasma mutant p53 protein and anti-p53 antibody expression in vinyl chloride monomer-exposed polyvinyl chloride workers. *Cancer Epidemiol Biomarkers Prev* 11:475–482.