Virchows Archiv A Pathol Anat (1992) 421:415–420

Virchows Archiv A
Pathological Anatomy and Histopathology

Low *p53* protein expression in salivary gland tumours compared with lung carcinomas

Ylermi Soini [1], Dia Kamel [1], Kyösti Nuorva [1], David P. Lane [2], Kirsi Vähäkangas [3], and Paavo Pääkkö [1,4]

[1] Department of Pathology, University of Oulu, Oulu, Finland
[2] Cancer Research Campaign Laboratory, Medical Sciences Institute, University of Dundee, Dundee, UK
[3] Department of Pharmacology and Toxicology, University of Oulu, Oulu, Finland
[4] Päivärinne Hospital, Muhos, Finland

Received March 23, 1992 / Received after revision June 24, 1992 / Accepted June 25, 1992

Summary. Fifty-one salivary gland tumours (23 pleomorphic adenomas, 5 Warthin's tumours, 12 mucoepidermoid carcinomas, 7 adenoid cystic carcinomas, 3 undifferentiated carcinomas and 1 acinic cell tumour) and 27 lung carcinomas (18 squamous cell carcinomas, 6 adenocarcinomas and 3 small cell carcinomas) were analysed immunohistochemically for the expression of *p53* nuclear phosphoprotein. Eight out of 51 (16%) salivary gland tumours were *p53* positive. Three of these were benign and 5 malignant. All 3 benign salivary gland tumours were pleomorphic adenomas and expressed only occasional nuclear positivity with less than 1% of tumour cells positive. Of the 5 *p53*-positive malignant tumours, 3 were mucoepidermoid carcinomas and 2 undifferentiated carcinomas. The malignant salivary gland tumours expressed more than 1% of positive nuclei in every case. Seventeen lung carcinomas were *p53* positive (63%). Thirteen of these were squamous cell carcinomas, 3 were adenocarcinomas and 1 small cell lung carcinoma. The results show that mutations of the *p53* gene may be infrequent in salivary gland tumours when compared with lung carcinomas. The relatively indolent course of some histological types of malignant salivary gland tumours could be associated with the preservation of the non-mutated *p53* gene in most of these tumours. The presence of *p53* positivity in some pleomorphic adenomas might, on one hand, suggest that *p53* gene alterations are also present in these tumours; on the other hand, the accumulation of the *p53* protein in these tumours might also be due to some unknown mechanism, not necessarily related to *p53* gene mutation.

Key words: Salivary gland – Salivary gland tumours – Oncogenes – *p53* – tumour suppressor genes

Correspondence to: Y. Soini, Department of Pathology, University of Oulu, Kajaanintie 52 D, SF-90220 Oulu, Finland

Introduction

Mutations of the tumour suppressor gene *p53* lead to neoplastic transformation of cells (Lavigueur et al. 1989; Nigro et al. 1989; Lane and Benchimol 1990). Changes of *p53* have been described in a wide variety of different tumours (Nigro et al. 1989; Hollstein et al. 1991) including different types of carcinomas (Cattoretti et al. 1988; Baker et al. 1989; Vogelstein 1989; Chiba et al. 1990; Iggo et al. 1990; Prosser et al. 1990; Thompson et al. 1990; Campo et al. 1991; Purdie et al. 1991), sarcomas (Masuda et al. 1987; Mulligan et al. 1990; Stratton et al. 1990) and lymphomas (Farrell et al. 1991; Gaidano et al. 1991; Hollstein et al. 1991; Winman et al. 1991; Soini et al. 1992a). The incidence of *p53* alterations varies in different types of tumours (Hollstein et al. 1991). In carcinomas, high incidences are found, for example in lung (Chiba et al. 1990; Iggo et al. 1990) and colon carcinomas (Baker et al. 1989; Campo et al. 1991; Purdie et al. 1991), while lower incidences are present in prostate, breast and thyroid carcinomas (Cattoretti et al. 1988; Wright et al. 1991; Soini et al. 1992b).

The *p53* gene is located in the short arm of chromosome 17 (Miller et al. 1986). The gene encodes a nuclear phosphoprotein which takes part in the control of cell proliferation (Mercer et al. 1984; Deppert et al. 1990; Steinmeyer et al. 1990; Milner 1991). The *p53* protein is associated with the cell cycle kinase $p34^{cdc2}$ (Bischoff et al. 1990) and may play a role in regulating the onset of DNA replication at the G1–S boundary (Wagner et al. 1991).

Mutations of the *p53* gene occur mainly in exons 5–8 (Hollstein et al. 1991). The mutated *p53* protein may form complexes with the wild-type *p53* protein and inactivate it (Iggo et al. 1990). The half-life of the mutated *p53* protein is longer than that of the wild-type protein (Finlay et al. 1988) and mutations of the *p53* gene often lead to an accumulation of mutated protein in the cells. This can be identified immunohistochemically by demonstrating reactivity for the protein in the nuclei of the neoplastic cells (Iggo et al. 1990; Bartkova et al. 1991).

Table 1. Clinical data of the cases and results of *p53* staining

Case	Sex	Age	Location	*p53*
Benign tumours				
Pleomorphic adenomas				
1.	F	35	Parotid gland	−
2.	M	43	Parotid gland	−
3.	F	35	Submandibular gland	−
4.	M	29	Parotid gland	−
5.	F	45	Parotid gland	−
6.	F	21	Parotid gland	−
7.	F	73	Parotid gland	−
8.	F	33	Parotid gland	−
9.	F	13	Parotid gland	−
10.	F	63	Parotid gland	−
11.	M	70	Parotid gland	−
12.	F	55	Parotid gland	−
13.	F	18	Parotid gland	(+)
14.	F	20	Submandibular gland	(+)
15.	F	35	Parotid gland	−
16.	F	44	Submandibular gland	−
17.	M	48	Parotid gland	−
18.	F	79	Parotid gland	−
19.	F	25	Parotid gland	−
20.	F	61	Parotid gland	−
21.	F	39	Parotid gland	(+)
22.	F	32	Parotid gland	−
23.	M	32	Parotid gland	−
Warthin's tumours				
24.	M	54	Parotid gland	−
25.	M	66	Parotid gland	−
26.	M	69	Parotid gland	−
27.	M	50	Parotid gland	−
28.	M	55	Parotid gland	−

F, Female; M, male

Quantification of the *p53* immunostaining: −, negative; (+), <1 of cells positive; +, 1–5% of cells positive; ++, 6–10% of cells positive; +++, 11–40% of cells positive; ++++, >40% of cells positive

Table 2. Clinical data of the cases and results of the *p53* staining

Case	Sex	Age	Location	*p53*
Malignant tumours				
Adenoid cystic carcinomas				
1.	M	47	Parotid gland	−
2.	M	55	Submandibular gland	−
3.	M	47	Parotid gland	−
4.	F	39	Submandibular gland	−
5.	M	31	Parotid gland	−
6.	M	55	Parotid gland	−
7.	M	55	Submandibular gland	−
Acinic cell tumours				
8.	F	70	Parotid gland	−
Mucoepidermoid carcinomas				
9.	F	45	Parotid gland	−
10.	F	40	Parotid gland	−
11.	M	69	Submandibular gland	−
12.	M	85	Parotid gland	−
13.	F	71	Parotid gland	−
14.	M	32	Parotid gland	−
15.	M	80	Parotid gland	+
16.	M	71	Parotid gland	+
17.	F	28	Submandibular gland	−
18.	F	40	Parotid gland	−
19.	F	69	Submandibular gland	−
20.	M	67	Parotid gland	++++
Undifferentiated carcinomas				
21.	M	75	Submandibular gland	−
22.	M	71	Parotid gland	++
23.	M	74	Parotid gland	++

F, Female; M, male

Quantification of the *p53* immunostaining as in Table 1

Immunoreactivity for the *p53* protein has been shown in a wide variety of human neoplasms (Cattoretti et al. 1988; Iggo et al. 1990; Bartek et al. 1991; Soini et al. 1992b).

In this study we analysed the presence of accumulated *p53* protein in various benign and malignant salivary gland tumours immunohistochemically with a polyclonal antibody CM-1 raised against the wild-type *p53* protein. It also detects the mutated protein (Bartkova et al. 1991; Midgley et al. 1992). For comparison, we also studied the expression of *p53* in 27 lung carcinomas. In order to explore the effect of the fixation time on the immunohistochemical results we examined three lung carcinoma cell lines (A-427, A-549, SK-MES-1) with 1, 2, 4 and 8 days' formalin fixation.

Materials and methods

Fifty-one benign and malignant salivary gland tumours and 27 malignant lung tumours were selected from the files of the Department of Pathology, Oulu University Central Hospital. The salivary gland tumours originated from years 1985–1991 and the lung tumours were from 1986. All the material had been fixed in 10% buffered formalin and embedded in paraffin. The salivary gland tumours were classified according to the World Health Organization's international classification of salivary gland tumours (WHO 1991) and the lung tumours according to the World Health Organization's international classification of lung tumours (WHO 1981). The diagnosis of all the cases was based on a light microscopic examination using the haematoxylin-eosin and alcian blue/periodic acid-Schiff stain. In some lung tumours, Grimelius stain was also used.

The salivary gland tumour material consisted of 23 pleomorphic adenomas, 5 Warthin's tumours, 12 mucoepidermoid carcinomas (6 well, 2 moderately and 4 poorly differentiated), 3 undifferentiated carcinomas, 1 acinic cell tumour (well differentiated) and 7 adenoid cystic carcinomas (5 cribriform, 1 tubular, 1 solid). The clinical data of the cases is shown in Tables 1 and 2.

The lung tumour material consisted of 18 squamous cell carcinomas, 6 adenocarcinomas (2 solid-type adenocarcinomas, 2 papillary adenocarcinomas and 2 bronchiolo-alveolar carcinomas) and 3 small cell carcinomas.

The immunostaining procedure using the antibody to *p53* protein was done according to Midgley et al. (1992). Briefly, 5-μm-thick sections were cut from the specimens and placed on slides coated with poly-L-lysine solution (Sigma, St. Louis, Mo., USA). The specimens were then dewaxed in xylene and rehydrated in graded alcohol. The endogenous peroxidase was blocked by immersing the sections for 20 min in 0.1% hydrogen peroxide in abso-

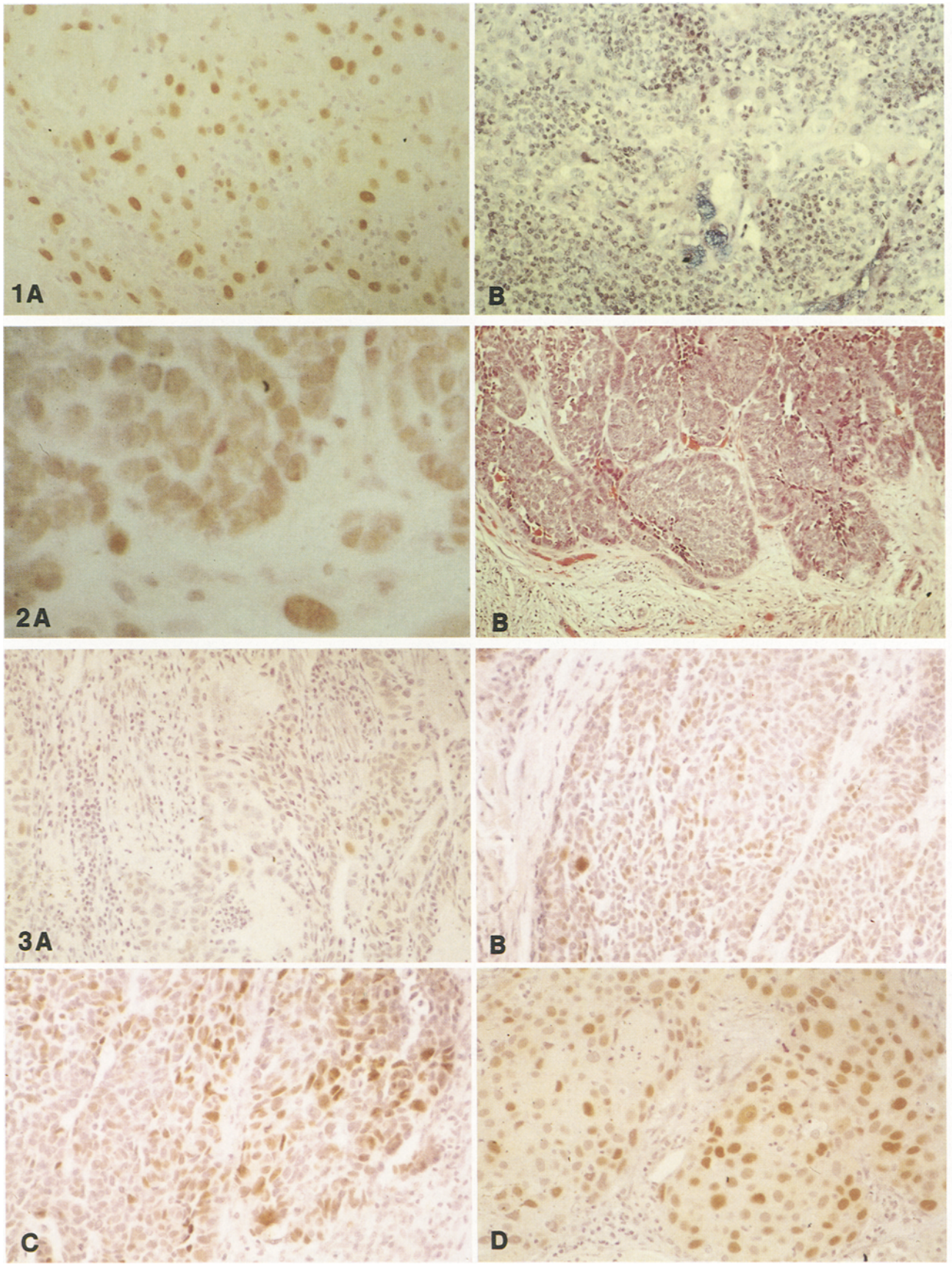

Fig. 1. **A** In a poorly differentiated mucoepidermoid carcinoma, tumour cell nuclei stain strongly for the antibody to the *p53* protein; immunoperoxidase stain, ×200. **B** In an alcian-blue stain mucus differentiation can be observed in this area. Alcian-blue stain, ×200

Fig. 2. **A** In an undifferentiated carcinoma of the salivary gland *p53* positivity can be observed in the nuclei of the neoplastic cells; immunoperoxidase stain, ×400. **B** A section of the tumour stained with haematoxylin-eosin, ×100

Fig. 3. Areas of lung squamous cell carcinomas showing weak + (**A**), moderate ++ (**B**), strong +++ (**C**) or very strong ++++ (**D**) *p53* immunoreactivity. Immunoperoxidase stain, ×200

lute methanol. The non-specific binding was blocked by incubating the slides in 20% fetal calf serum in phosphate buffered saline (PBS) for 20 min.

In the immunostaining the avidin-biotin complex method was used (Hsu et al. 1981). The sections were first incubated overnight at 4° C with a primary polyclonal rabbit *p53* antibody CM-1 with a dilution of 1:1000 (Midgley et al. 1992) followed by a secondary biotinylated anti-rabbit antibody (dilution 1:100) (Dakopatts, Copenhagen, Denmark) and the avidin-biotin complex (Dakopatts). Careful rinses were done with several changes of PBS between each stage of the procedure. The colour was developed with diaminobenzidine, whereafter the sections were lightly counterstained with haematoxylin and mounted with Eukitt (Kindler, Freiburg, FRG).

Negative controls for the immunostaining were carried out by substituting the primary antibody with PBS or with non-immune rabbit serum.

The results of *p53* reactivity were evaluated quantitatively and divided into six groups [−, negative; (+), <1% of cell positive; +, 1–5% of cells positive; ++, 6–10% of cells positive; +++, 11–40% of cells positive; ++++, >40% of cells positive] according to the estimated number of positive cells (see Fig. 3). Only nuclear staining was interpreted as positive.

Three undifferentiated human lung carcinoma cell lines (A-427, A-549, SK-MES-1), previously known to express *p53* positivity (Caamano et al. 1991), were obtained from the American Type Culture Collection (ATCC, Rockville, Md., USA) and grown as recommended. The cultured cells were fixed in 10% buffered formalin for 1, 2, 4 and 8 days after which the samples were cytocentrifuged and placed into melted agar which was then allowed to solidify. The samples were then embedded in paraffin, sectioned and immunostained as described above. The results were evaluated by counting the proportion of positive nuclei from 500 cells of each sample.

Results

The results of the *p53* immunostaining in salivary gland tumours are shown in Tables 1 and 2. Eight of 51 (16%) salivary gland tumours showed *p53* protein expression. Three of 28 (11%) benign salivary gland tumours were *p53* positive. All 3 cases were pleomorphic adenomas. They contained less than 1% of positively stained cells in each case. Five of 23 (22%) malignant salivary gland tumours were *p53* positive. Three of 14 mucoepidermoid carcinomas (21%) contained tumour cell nuclei which stained positive for *p53* (Fig. 1). All 3 cases were poorly differentiated. In 2 tumours the proportion of *p53* positive cells was approximately 10%, while in the third tumour, about 60% of the tumour cells were *p53* positive. The positivity was mainly located in poorly differentiated cells. However, in the mucoepidermoid carcinoma with 60% of cells positive the squamous cell component also showed *p53* positivity. Additionally, 2 undifferentiated salivary gland tumours were *p53* positive (Fig. 2). The proportion of positive cells was approximately 30–40% in both cases.

The results of the *p53* immunostaining in lung carcinomas are shown in Table 3. Seventeen lung carcinomas were *p53* positive (63%). The positive cases consisted of 13 squamous cell carcinomas (72% of all squamous cell carcinomas) (Fig. 3), 3 adenocarcinomas (50% of all adenocarcinomas) and 1 small cell carcinoma (33% of all small cell carcinomas). Seven of 8 poorly differentiated (grade III) carcinomas (88%), 5 of 8 moderately differentiated (grade II) (63%) and 4 of 8 well-differentiated (grade I) tumours (50%) were *p53* positive. No significant association was found between tumour size, lymph node metastases, the age of the patients and *p53* positivity. There were slightly more *p53*-positive cases in grade III than in grade I–II carcinomas. On average, *p53*-positive squamous cell carcinomas contained more *p53*-positive cells than adenocarcinomas.

The results of the *p53* protein immunostaining of the three lung carcinoma cell lines are presented in Table 4.

Table 3. Clinical data and results of the *p53* staining in lung tumours

Case	Age[a]	Grade	*p53*
Squamous cell carcinomas:			
1.	59	III	++
2.	65	III	++
3.	45	III	++
4.	68	III	+++
5.	57	III	++
6.	33	III	+++
7.	69	II	++++
8.	66	II	++
9.	74	II	−
10.	73	II	++
11.	67	II	++
12.	46	II	−
13.	61	I	−
14.	68	I	++
15.	64	I	+++
16.	73	I	−
17.	79	I	−
18.	63	I	++
Adenocarcinomas:			
19.	55	III	+
20.	62	III	−
21.	73	II	−
22.	68	II	++
23.	36	I	++
24.	74	I	−
Small cell carcinomas:			
25.	61	−	++++
26.	68	−	−
27.	65	−	−

[a] All patients were male

Quantification of the *p53* immunostaining as in Table 1

Table 4. *p53* staining in cell lines

	Percentage of positive cells[a]		
	A-427	A-549	SK-MES-1
Fixation time			
1 day	1%	9.8%	0%
2 days	11.2%	17.6%	0%
4 days	22%	7.3%	0%
8 days	13.5%	3%	0%

[a] Five hundred cells of each sample were analysed for the proportion of *p53* nuclear positivity

Two cell lines (A-427 and A-549) expressed *p53*-positive cells while one (SK-MES-1) was negative. Quantitatively and qualitatively the staining was strongest after 4 days' fixation for A-427 and after 2 days' fixation for A-549 with 22% and 17.6% of *p53*-positive cells, respectively.

Discussion

Our results show that the incidence of *p53* positivity in salivary gland tumours is low when compared with lung tumours. Only 8 salivary gland tumours were *p53* positive while 17 lung carcinomas expressed the *p53* protein. If only malignant salivary gland tumours are considered, the incidence is still lower (22%) than in lung carcinomas (63%).

The results add further evidence to previous observations that there are differences in the expression of *p53* in tumours of different location and type (Cattoretti et al. 1988; Iggo et al. 1990; Purdie et al. 1991; Wright et al. 1991; Soini et al. 1992b). The reason for the variability between different types of tumours is obscure. However, it seems that hormone-sensitive tumours are less *p53* positive (Cattoretti et al. 1988; Wright et al. 1991; Soini et al. 1992b). In fact, it has been shown that there is an inverse relationship between the oestrogen receptor status and *p53* positivity in breast carcinomas (Cattoretti et al. 1988). However, a high percentage of *p53* positivity is found in tumours apparently related to chemical carcinogens (such as lung carcinomas).

In our previous investigations we found that *p53* positivity is more frequently present in carcinomas with a low degree of differentiation (Soini et al. 1992b). Since 2 out of 3 undifferentiated salivary gland carcinomas were *p53* positive and all three *p53*-positive mucoepidermoid carcinomas represented poorly differentiated tumours, the *p53* positivity in malignant salivary gland tumours also tends to concentrate in neoplasms which are less differentiated and biologically more aggressive. Furthermore, the low incidence of *p53* positivity could be reflected in the biological behaviour of malignant salivary gland tumours. Acinic cell tumours, adenoid cystic carcinomas and mucoepidermoid carcinomas all are relatively slowly growing tumours with a relatively good prognosis (Thackray and Lucas 1974). It may be that the preservation of a non-mutated *p53* gene hinders the proliferation of the neoplastic cells in these neoplasms, leading to a less aggressive behaviour.

Of the 28 benign salivary gland tumours 3 pleomorphic adenomas expressed occasional *p53* positivity with a frequency of less than 1% of cell nuclei positive. Similar reactivity has been observed in benign tumours of the soft tissues (Soini et al. 1992c). The presence of *p53* positivity in benign tumors may suggest that accumulation of the *p53* protein is not necessarily associated with *p53* gene alterations. In fact, it has been shown that phytohaemagglutinin-stimulated rapidly proliferating lymphocytes may express detectable levels of *p53* protein (Mercer and Baserga 1985). It may also be possible that benign tumours contain *p53* mutations and there is a previous report of a benign meningioma with a *p53* mutation (Mashiyama et al. 1991).

Even though pleomorphic adenomas contain *p53*-positive cells, the results show that there is a quantitative difference in *p53* positivity between benign and malignant *p53*-positive salivary gland tumours. Though this might be used as an adjunct in the assessment of the biological behaviour of salivary gland tumours, certain caution is needed. Strong *p53* immunoreactivity in tumours most certainly speaks in favour of malignancy.

From previous investigations, the incidence of *p53* positivity in lung carcinomas is about 40–60% (Iggo et al. 1990; Soini et al. 1992b). This is in accordance with our results. The incidence is higher in squamous cell carcinomas than in other histological types (Iggo et al. 1990; Soini et al. 1992b), a finding also observed in our material. In previous reports, an association between the grade of the tumour and *p53* positivity has been found (Soini et al. 1992b) and the incidence of *p53* positivity was higher in grade III tumours than in grade I–II tumours in our material.

In order to assess the effect of the time of formalin fixation on the immunohistochemical staining results we tested *p53* immunoreactivity in three lung carcinoma cell lines (A-427, A-549, SK-MES-1) which have previously been shown to be *p53* positive (Caamano et al. 1991). Two (A-427, A-549) of these cell lines showed *p53* immunoreactivity with the CM-1 antibody. Assessed by the proportion of positive cells, the most intense reaction was found after 2–4 days' fixation (see Table 4), the fixation time which is used in our laboratory for surgical pathology samples. The fixation time is thus optimal for the material used in this study. The fact that one lung carcinoma cell line (SK-MES-1) was negative is probably due to the fact that these authors used different *p53* antibodies (Caamano et al. 1991).

In conclusion, our results show that *p53* mutations, which are reflected by *p53* immunoreactivity in the nuclei of neoplastic cells, are relatively infrequent in salivary gland tumours. In malignant salivary gland tumours the *p53* positivity tends to be more evident in less differentiated tumours. The relatively benign behaviour of acinic cell, adenoid cystic and mucoepidermoid tumours could be due to the fact that most of these tumours still contain a non-mutated *p53* gene. Since pleomorphic adenomas may occasionally contain *p53*-positive cells, *p53* immunoreactivity in salivary gland tumours cannot be used as a direct marker of malignancy. Strong *p53* positivity in salivary gland tumours favours a malignant diagnosis, however.

Acknowledgements. This study was supported by the Finnish Cultural Fund and the Finnish Anti-Tuberculosis Association.

References

Baker SJ, Fearon EJ, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, Tuinen P van, Ledbetter DH, Barker DF, Nakamura Y, White R, Vogelstein B (1989) Chromosome 17 deletions and *p53* gene mutations in colorectal carcinomas. Science 244:217–221

Bartek J, Bartkova J, Vojtesek B, Staskova Z, Lukas J, Rejthar A, Kovarik J, Midgley CA, Gannon JV, Lane DP (1991) Aberrant expression of the *p53* oncoprotein is a common feature of a wide spectrum of human malignancies. Oncogene 6:1699–1703

Bartkova J, Bartek J, Lukas J, Voitesek B, Staskova Z, Rejthar A, Kovarik J, Midgley CA, Lane DP (1991) *p53* protein alterations in human testicular cancer including pre-invasive intratubular germ-cell neoplasia. Int J Cancer 49:196–202

Bischoff JR, Friedman PN, Marshak DR, Prives C, Beach D (1990) Human *p53* is phosphorylated by p64-cdc2 and cyclin B-cdc2. Proc Natl Acad Sci USA 87:4766–4770

Caamano J, Ruggeri B, Momiki S, Sickler A, Zhang SY, Klein-Szanto AJP (1991) Detection of *p53* in primary lung tumors and nonsmall cell lung carcinoma cell lines. Am J Pathol 139:839–845

Campo E, Calle-Martin O de la, Miguel R, Palacin A, Romero M, Fabregat V, Vivers J, Cardesa A, Yague J (1991) Loss of heterozygosity of *p53* gene and *p53* protein expression in human colorectal carcinomas. Cancer Res 51:4436–4442

Cattoretti G, Rilke F, Andreola S, D'Amato L, Delia D (1988) *p53* expression in breast cancer. Int J Cancer 41:178–183

Chiba I, Takahashi T, Nau MM, D'Amico D, Curiel DT, Mutsudomi T, Buchhagen DL, Carbone D, Piantadosi S, Koga H, Reissman PT, Slamon DJ, Holmes EC, Minna JD (1990) Mutations in the *p53* gene are frequent in primary, resected non-small cell lung cancer. Oncogene 5:1603–1610

Deppert W, Buschhausen-Denker G, Patschinsky T, Steinmeyer K (1990) Cell cycle control of *p53* in normal (3T3) and chemically transformed (Meth A) mouse cells. II. Requirement for cell cycle progression. Oncogene 5:1701–1706

Farrell PJ, Allan GJ, Shanahan F, Vousden KH, Crook T (1991) *p53* is frequently mutated in Burkitt's lymphoma cell lines. EMBO J 10:2879–2887

Finlay CA, Hinds PW, Tan T-H, Eliyahu D, Oren M, Levine J (1988) Activating mutations for transformation by *p53* produce a gene product that forms an hsc70-*p53* complex with an altered half-life. Mol Cell Biol 8:531–539

Gaidano G, Ballerini P, Gong JZ, Inghirami G, Neri A, Newcomb EW, Magrath IT, Knowles DM, Dalla-Favera R (1991) *p53* mutations in human lymphoid malignancies: association with Burkitt lymphoma and chronic lymphocytic leukemia. Proc Natl Acad Sci USA 88:5413–5417

Hollstein M, Sidransky D, Vogelstein B, Harris C (1991) *p53* mutations in human cancers. Science 253:49–53

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Iggo R, Gatter K, Bartek J, Lane D, Harris AL (1990) Increased expression of mutant forms of *p53* oncogene in primary lung cancer. Lancet 335:675–679

Lane D, Benchimol S (1990) *p53*: oncogene or anti-oncogene. Gene 4:1–8

Lavigueur A, Maltby V, Mock D, Rossant J, Pawson T, Bernstein A (1989) High incidence of lung, bone, and lymphoid tumours in transgenic mice overexpressing mutant alleles of the *p53* oncogene. Mol Cell Biol 9:3989–3991

Mashiyama S, Murakami Y, Yoshimoto T, Sekiya T, Hayashi K (1991) Detection of *p53* gene mutations in human brain tumors by single-strand conformation polymorphism analysis of polymerase chain reaction products. Oncogene 6:1313–1318

Masuda H, Miller C, Koeffler HP, Battifora H, Cline MJ (1987) Rearrangement of the *p53* gene in human osteogenic sarcomas. Proc Natl Acad Sci USA 84:7716–7719

Mercer WE, Baserga R (1985) Expression of the *p53* protein during the cell cycle of human peripheral blood lymphocytes. Exp Cell Res 160:31–46

Mercer WE, Avignolo C, Basegra R (1984) Role of *p53* protein in cell proliferation as studied by microinjection of monoclonal antibodies. Mol Cell Biol 4:276–281

Midgley CA, Fisher CJ, Bartek J, Vojtesek B, Lane D, Barnes DM (1992) Expression of human *p53* in bacteria: application to the analysis of *p53* expression in human tumours. J Cell Sci 101:183–189

Miller C, Mohandas T, Wolf D, Prokocimer M, Rotter V, Koeffler HP (1986) Human *p53* gene localized to short arm of chromosome 17. Nature 319:783–784

Milner J (1991) The role of *p53* in normal control of cell proliferation. Curr Opin Cell Biol 3:282–286

Mulligan LM, Matlashewski GJ, Scrable HJ, Cavenee WK (1990) Mechanisms of *p53* loss in human sarcomas. Proc Natl Acad Sci USA 87:5863–5867

Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hostetter R, Cleary K, Bigner SH, Davidson N, Baylin S, Devilee P, Glover T, Collins FS, Weston A, Modali R, Harris CC, Vogelstein B (1989) Mutations in the *p53* gene occur in diverse human tumour types. Nature 342:705–708

Prosser J, Thompson AM, Cranston G, Evans HJ (1990) Evidence that *p53* behaves as a tumour suppressor gene in sporadic breast tumours. Oncogene 5:1573–1579

Purdie CA, O'Grady J, Piris J, Wyllie AH, Bird CC (1991) *p53* expression in colorectal tumors. Am J Pathol 138:807–813

Soini Y, Pääkkö P, Alavaikko M, Vähäkangas K (1992a) *p53* expression in lymphatic malignancies. J Clin Pathol 45 (in press)

Soini Y, Pääkkö P, Nuorva K, Kamel D, Lane DP, Vähäkangas K (1992b) Comparative analysis of *p53* protein immunoreactivity in prostatic, lung and breast carcinomas. Virchows Arch [A] (in press)

Soini Y, Vähäkangas K, Nuorva K, Kamel D, Lane DP, Pääkkö P (1992c) *p53* immunohistochemistry in malignant fibrous histiocytomas and other mesenchymal tumours. J Pathol 168 (in press)

Steinmeyer K, Maacke H, Deppert W (1990) Cell cycle control by *p53* in normal (3T3) and chemically transformed (Meth A) mouse cells. I. Regulation of *p53* expression. Oncogene 5:1691–1699

Stratton MR, Moss S, Warren W, Patterson H, Clark J, Fisher C, Fletcher CDM, Ball A, Thomas M, Gusterson BA, Cooper CS (1990) Mutation of the *p53* gene in human soft tissue sarcomas: association with abnormalities of the *RB1* gene. Oncogene 5:1297–1301

Thackray AC, Lucas RB (1974) Tumors of the major salivary glands. Atlas of tumor pathology, 2nd series, fascicle 10. Armed Forces Institute of Pathology, Bethesda, pp 69–99

Thompson AM, Steel CM, Chetty U, Hawkins RA, Miller WR, Carter DC, Forrest APM, Evans HJ (1990) *p53* gene mRNA expression and chromosome *17p* allele loss in breast cancer. Br J Cancer 61:74–78

Vogelstein B (1989) Chromosome *17* deletions and *p53* gene mutations in colorectal carcinomas. Science 244:217–221

Wagner P, Simanis V, Maimets T, Keenan E, Addison C, Brain R, Grimaldi M, Sturzbecher HW, Jenkins J (1991) A human tumour-derived mutant *p53* protein induces a $p34^{cdc2}$ reversible growth arrest in fission yeast. Oncogene 6:1539–1547

Winman KG, Magnusson KP, Ramqvist T, Klein G (1991) Mutant *p53* detected in a majority of Burkitt lymphoma cell lines by monoclonal antibody PAb240. Oncogene 6:1633–1639

World Health Organization (1981) Histological typing of lung tumours. International classification of tumours no. 1. World Health Organization, Geneva

World Health Organization (1991) Histological typing of salivary gland tumours. International histological classification of tumours no. 7, 2nd edn. World Health Organization, Geneva

Wright PA, Lemoine NR, Goretzki PE, Wyllie FS, Bond J, Hughes C, Röher H-D, Williams ED, Wynford-Thomas D (1991) Mutations of the *p53* gene in a differentiated human thyroid carcinoma cell line, but not in primary thyroid tumors. Oncogene 6:1693–1697