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Comparative analysis of *p53* protein immunoreactivity in prostatic, lung and breast carcinomas

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Summary. In this study we analysed the expression of *p53* protein in a total of 143 carcinomas immunohistochemically. These consisted of 34 prostatic adenocarcinomas, 59 lung and 50 breast carcinomas. In 28 cases, an average of 2–3 additional sections from different tumour areas were analysed. Forty-nine of the 143 carcinomas (34%) showed typical nuclear immunoreactivity by immunohistochemical staining with the *p53* antibody CM-1. Two of the 34 prostatic carcinomas (6%) were *p53* positive while 25 of the 59 lung carcinomas (43%) and 22 of the 50 breast carcinomas (44%) showed positivity for *p53*. By grade: 49% of grade III tumours, 36% of grade II and 5% of grade I tumours were *p53* positive. There were significantly more *p53*-positive cases in grade II–III tumours than in grade I tumours ($P=0.001$) when all tumours were taken into account. Further, there were significantly more *p53*-positive cases in grade III than in grade I–II tumours ($P=0.001$). In lung tumours there were significantly more *p53*-positive cases in grade II–III tumours than in grade I tumours ($P=0.018$). Similarly, there were significantly more *p53*-positive tumours in grade III breast tumours than in grade I–II tumours ($P=0.003$). The low incidence of *p53* positivity in prostate carcinomas suggests that mutations of the *p53* gene are not as frequent in the neoplastic transformation of these tumours as in lung or breast carcinomas. The association of *p53* positivity with tumours of higher grade suggests that *p53* mutations lead to tumours of a more aggressive type. The analysis of tumours by multiple sections indicates that *p53* positivity is not evenly distributed in tumour tissue. Therefore, analysis of additional tumour areas may reveal positivity some cases, which is not evident if only one section is studied.

Key words: *p53* – Oncogenes – Carcinoma – Prostate gland – Lung – Breast

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Introduction

The tumour suppressor gene *p53* was originally considered to be an oncogene (Eliyahu et al. 1984; Parada et al. 1984). However, it was soon discovered that the *p53* gene itself has tumour suppressor properties and that these properties are lost as a consequence of mutations in the gene (Eliyahu et al. 1989; Finlay et al. 1989).

It is located in the short arm of chromosome 17 (Miller et al. 1986). Mutations, which lead to a functional inactivation of the gene, are principically found in the exons 5–8 (Hollstein et al. 1991).

Mutations of the *p53* gene have been found in several types of carcinoma (Nigro et al. 1989; Hollstein et al. 1991) including colon (Baker et al. 1989; Campo et al. 1991; Purdie et al. 1991), breast (Cattoretti et al. 1988; Prosser et al. 1990; Thompson et al. 1990), ovary (Mazars et al. 1991), lung (Chiba et al. 1990; Iggo et al. 1990) and liver (Bressac et al. 1991; Hsu et al. 1991). *p53* mutations have also been found in different types of sarcomas (Masuda et al. 1987; Mulligan et al. 1990; Stratton 1990) and in lymphomas and Burkitt's lymphoma cell lines (Farrell et al. 1991; Gaidano et al. 1991; Hollstein et al. 1991). The widespread occurrence of *p53* gene mutations in different types of malignant tumours suggests that *p53* gene mutations have a crucial role in neoplastic transformation of many types of human neoplasia.

The mutated *p53* protein has a longer half-life than the wild-type protein (Finlay et al. 1988) and is able to complex with it, thus inactivating it (Iggo et al. 1990). The mutated *p53* protein also accumulates in the cells (Iggo et al. 1990; Bartkova et al. 1991; Midgley et al. 1992). Hence, mutations in this gene can be analysed immunohistochemically by detecting the accumulated *p53* protein in the cell nuclei (Iggo et al. 1990). The incidence of *p53* immunoreactivity in lung and colon carcinomas has been found to be 60–70% (Iggo et al. 1990; Campo et al. 1991; Purdie et al. 1991), while it is lower in breast and ovarian carcinomas (Cattoretti et al. 1988; Mazars et al. 1991). Some *p53* mutations do not lead to an accumulation of *p53* protein, however (Lehman

et al. 1991; Vähäkangas et al. 1992). *p53* mutations leading to a stop codon or deletions of the *p53* gene may lead to a situation where no protein can be found (Vähäkangas et al. 1992).

In order to study the frequency of *p53* positive cases in prostatic adenocarcinomas we here analysed 34 prostatic adenocarcinomas immunohistochemically using a polyclonal antibody raised against the wild type *p53* protein, which also detects the mutated *p53* protein (Bartkova et al. 1991; Midgley et al. 1992). Additionally, 59 lung and 50 breast carcinomas were studied.

Materials and methods

Thirty-four prostatic carcinomas, 59 cases of various lung carcinomas and 50 breast carcinomas were collected from the files of the Department of Pathology, Oulu University Central Hospital between 1979 and 1991. All the material used had been fixed in 10% neutral formalin and embedded in paraffin. All prostatic carcinomas were adenocarcinomas obtained by radical prostatectomy. Eight cases represented grade I, 17 grade II, and 9 grade III carcinomas. The diganosis of prostatic adenocarcinoma and tumour grades were based on the WHO classification of prostatic tumours according to Mostofi et al. (1980).

The lung carcinomas consisted of 34 squamous cell carcinomas of which 4 were well differentiated (grade I), 19 moderately differentiated (grade II) and 11 poorly differentiated (grade III), 3 small cell carcinomas, 21 adenocarcinomas of which 3 were well-differentiated (grade I), 6 moderately differentiated (grade II) and 12 poorly differentiated (grade III), and 1 moderately differentiated adenosquamous carcinoma (grade II). The breast tumours consisted of 42 infiltrative ductal carcinomas (4 grade I, 27 grade II and 11 grade III), 6 infiltrative lobular carcinomas and 2 mucinous carcinomas. The diagnosis of all the cases and the grades of the tumours were based on a light microscopic examination with a conventional haematoxylin and eosin stain according to the criteria of the World Health Organization (WHO 1981a, b). Some of the lung tumours were also stained with alcian blue periodic acid-Schiff and Grimelius stain.

For immunohistochemistry one section of each tumour was analysed. The immunostaining procedure was done according to Midgley et al. (1992). Sections 5 μm thick were cut from the paraffin blocks and placed on slides coated with poly-L-lysine solution (Sigma, St. Louis, Mo., USA). The specimens were then dewaxed in xylene and dehydrated in graded alcohol. The endogenous peroxidase was blocked by immersing the sections for 10 min in 0.1% hydrogen peroxide in absolute methanol. The non-specific binding was blocked by incubating the slides in 20% fetal calf serum in phosphate-buffered saline (PBS) for 20 min.

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, prepared against human wild-type *p53* protein in a recombinant bacterial system and fully characterized by Midgley et al. (1992) and Barkova et al. (1991), followed by a secondary biotinylated anti-rabbit antibody (dilution 1:400) and the avidin-biotin complex (both from Dakopatts, Copenhagen, Denmark). 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 in Eukitt (Kindler, FRG).

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

In 11 prostate adenocarcinomas, 10 lung carcinomas and 7 breast carcinomas more than one section of the tumour was analysed. All the additional sections were made of samples from different areas of the tumours than the primary sections. On average, 2–3 sections from separate tumour areas were analysed in these cases (Table 1). Altogether 60 new sections were analysed.

Table 1. Cases with multiple sections

Primary sections	Additional sections				
Prostatic adenocarcinomas					
−	−				
−	−				
−	−	−	−		
−	−	−	−		
−	−				
−	−	−	−	−	
−	−				
−	−				
−	−				
−	−				
−	−				
Lung carcinomas					
+	+				
−	−	−	−	−	
−	−	−	−		
−	−	+	+	+	
−	−	−	−	−	−
−	−	−			
−	−	−	−	−	
−	−	−	−		
+	+	−			
+	+				
Breast carcinomas					
−	−	−	−		
−	−				
+	+	−	−		
−	−				
−	−				
−	−	−	−		
−	−				

+, *p53* positivity found in tumour cells; −, *p53* positivity not found in tumour cells

The results were evaluated quantitatively and divided into five groups (−, negative; +, <5% of cells positive; ++, 6–10% of cells positive; +++, 11–40% of cells positive; ++++, >40% of cells positive). Only nuclear staining was interpreted as positive. The significance of the associations was determined using either the chi-squared or Fisher's exact probability test.

Results

The results of the immunostaining for the prostatic, lung and breast carcinomas are presented in Tables 2 and 3. Forty-nine of 143 (34%) carcinomas were *p53* positive. Of 132 graded tumours, 21 *p53*-positive cases were of grade III (49% of all grade III tumours), 25 of grade II (36% of all grade II tumours) and 1 of grade I (5% of all grade I tumours). There were significantly more *p53*-positive cases in grade II–III tumours than in grade I tumours ($P=0.001$). Furthermore, there were significantly more *p53*-positive cases in grade III tumours than in grade I–II tumours ($P=0.001$).

Table 2. *p53* staining in prostate, lung and breast carcinomas

Histological type	Frequency of *p53* cases/total number of cases	Percentage of *p53* positive cases
Prostatic carcinomas		
Adenocarcinoma	2/34	6%
Total	2/34	6%
Lung carcinomas		
Squamous cell carcinoma	17/34	50%
Small cell lung carcinoma	1/3	33%
Adenocarcinoma	6/21	29%
Adenosquamous carcinoma	1/1	100%
Total	25/59	43%
Breast carcinomas		
Ductal carcinoma	22/42	52%
Lobular carcinoma	0/6	0%
Mucinous carcinoma	0/2	0%
Total	22/50	44%

Table 3. Number of *p53* positive cases in tumours of different grades

Grade	Prostate	Lung	Breast	Total
I	0/8 (0%)	0/7 (0%)	1/4 (25%)	1/19 (5%)
II	1/17 (6%)	13/26 (50%)	11/27 (41%)	25/70 (36%)
III	1/9 (11%)	10/23 (43%)	10/11 (91%)	21/43 (49%)
Total	2/34 (6%)	23/56 (41%)	22/42 (52%)	47/132 (38%)

Only 2 of the 34 prostatic adenocarcinomas (6%) expressed *p53*. One case expressed *p53* very strongly (Fig. 1), the other weakly. The case with strong *p53* expression was grade III and that with weak expression was grade II carcinoma. There was no association between the age of the patient, the metastatic status and the *p53* positivity (data not shown).

Twenty-five of 59 lung carcinomas (43%) expressed *p53*. Of the *p53*-positive lung carcinomas, 17 were squamous cell carcinomas (Fig. 2), 6 adenocarcinomas (2 papillary and 4 solid with mucus production), 1 small cell lung carcinoma and 1 an adenosquamous carcinoma.

Of the 17 *p53*-positive squamous cell carcinomas 6 represented poorly differentiated grade III and 11 moderately differentiated grade II tumours. Thus 55% of the grade III squamous cell carcinomas and 58% of the grade II squamous cell carcinomas were *p53* positive. Of the 6 positive adenocarcinomas, 4 represented grade III and 2 grade II tumours. Thus, 33% of the grade III adenocarcinomas and 33% of the grade II adenocarcinomas were *p53* positive. There were significantly more *p53*-positive cases in grade II–III lung tumours than in

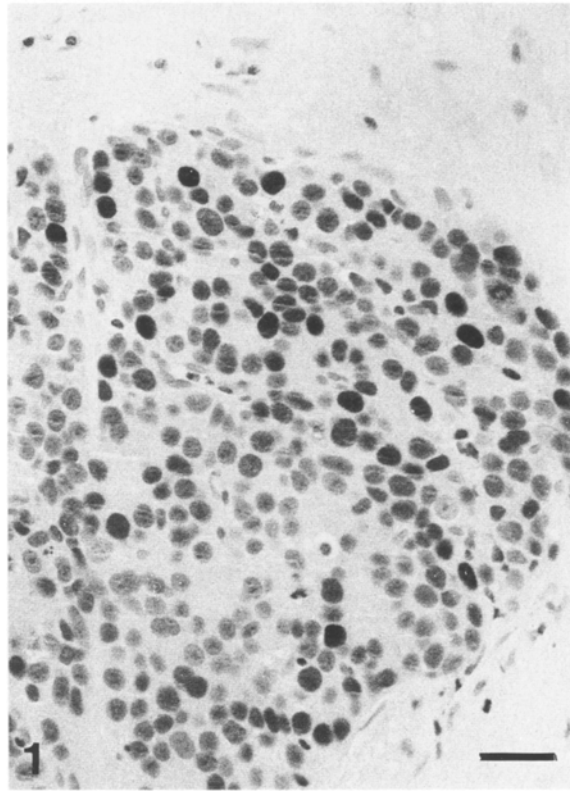

Fig. 1. In this case of prostate adenocarcinoma, strong nuclear *p53* staining can be seen in the nuclei of the tumour cells. *Scale bar* = 39 μm; × 260

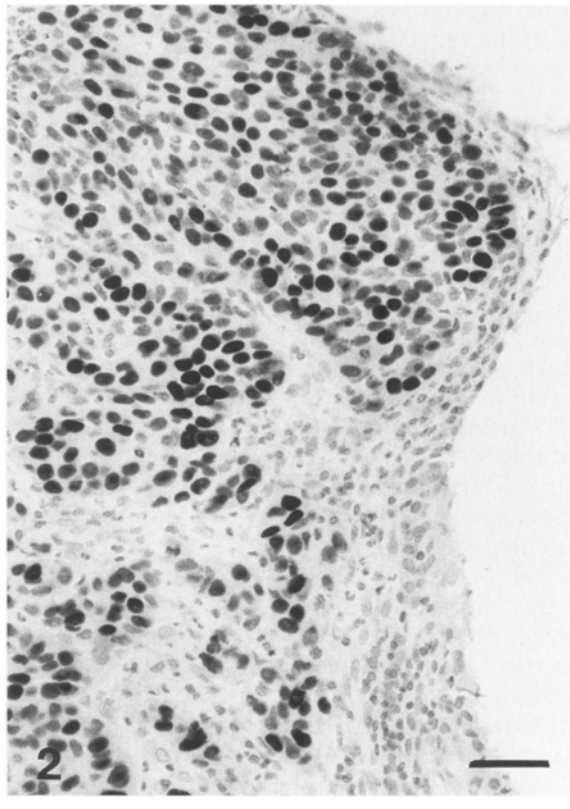

Fig. 2. In a squamous cell carcinoma of the lung, the tumour cells show strong *p53* staining. *Scale bar* = 39 μm; × 260

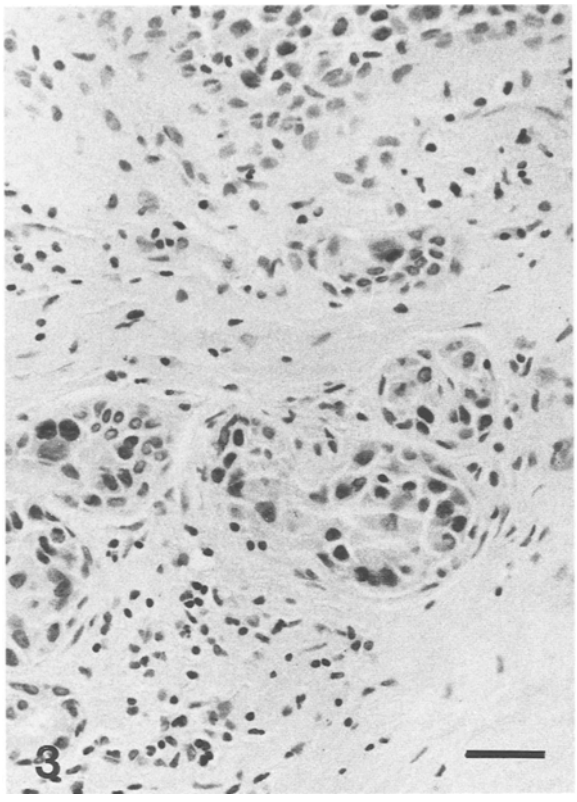

Fig. 3. In a ductal adenocarcinoma of portion of the tumour cell nuclei are positive for *p53*. *Scale bar* = 39 μm; ×260

grade I tumours ($P=0.018$). There was no association between the tumour size, the age of the patients or the existence of hilar metastases and the *p53* positivity.

Twenty-two of 50 (44%) breast carcinomas expressed *p53*. Ten of 11 grade III ductal carcinomas were *p53* positive, while 11 of 27 grade II tumours and 1 of 4 grade I tumours were *p53* positive (Fig. 3). There were significantly more *p53*-positive cases in grade III carcinomas than in grade I–II carcinomas ($P=0.003$). There was no association between the age of the patients, the metastatic status and the *p53* positivity. All 6 lobular and 2 mucinous carcinomas were *p53* negative.

In additional sections none of the 11 prostate tumours were positive in the primary immunostaining or in the additional sections. Three of 10 lung tumours and 1 of 7 breast tumours were positive in primary immunostaining. Of the 10 lung carcinomas, 1 squamous carcinoma, in which no *p53* positivity had been shown in the primary immunostaining, was found to be positive for *p53*. Three of the 10 cases which were *p53* positive in the primary immunostaining were also positive in all but 1 of the additional sections. In the 7 breast carcinomas studied, no new *p53*-positive cases were found. In 1 *p53*-positive case, the positivity could be verified in 1 of 3 sections.

No *p53* staining could be observed in the nuclei of the surrounding non-neoplastic tissues. In 1 lung tumour sample, severely dysplastic bronchial squamous cell epithelium could be seen in association with the lung tumour and a few of the nuclei of the dysplastic epithelium stained positive for *p53*. In all cases studied the mastopathic epithelium in breast tissue and hyperplastic prostatic epithelium showed no *p53* positivity.

Discussion

In this study we analysed the expression of *p53* protein in 34 prostatic, 59 lung and 50 breast carcinomas immunohistochemically by using a polyclonal antibody to *p53* protein. The results show that there is an association between high tumour grade and *p53* positivity and that *p53* is expressed in prostatic carcinomas with a lower frequency than in lung or breast carcinomas.

Overall, 49% of the grade III, 36% of the grade II and 5% of grade I tumours expressed *p53*. There was a correlation between *p53* positivity and high tumour grade regardless of the histological type of the tumour. An association between high tumour grade and *p53* positivity has also been found previously in breast carcinoma (Cattoretti et al. 1988; Midgley et al. 1992). The association of p53 positivity and high tumour grade suggests that *p53* mutations lead preferentially to more aggressive types of tumours.

In a recent study, *p53* gene mutations were found in 3 of 5 prostate carcinoma cell lines (Isaacs et al. 1991), indicating a higher incidence of *p53* gene mutations in prostate carcinoma than our results suggest. However, it has been suggested that *p53* mutations may facilitate the growth of carcinoma cells in culture (Wright et al. 1991). In line with this Burkitt's lymphoma cell lines harbour *p53* mutations more frequently than the material obtained from clinical cases (Farrell et al. 1991; Gaidano et al. 1991). Such a phenomenon could also explain the higher frequency of *p53* mutations in prostate cell lines.

However, it has been found that not all *p53* mutations lead to an accumulation of the *p53* protein and thus cannot be detected by immunohistochemistry (Lehman et al. 1991; Vähäkangas et al. 1992). Consequently, our material shows only cases in which accumulation of mutated *p53* protein has occurred.

The low incidence of *p53*-positive cases in prostate carcinoma indicates that point mutations of *p53* gene leading to an accumulation of the mutated protein are not frequent in the neoplastic transformation of prostate epithelial cells. In previous immunohistochemical investigations variations in the incidence of *p53* positivity have been observed in different types of carcinomas. The incidence of *p53* positivity seems to be high in lung and colon carcinomas (Iggo et al. 1990; Purdie et al. 1991), while lower incidences are found in breast, ovarian or thyroid carcinomas (Cattoretti et al. 1988; Mazars et al. 1991; Wright et al. 1991). The lower incidence of *p53* positivity in prostate adenocarcinomas might be related to the fact that prostate tumours are hormone sensitive. In breast carcinomas, it has been shown that the positive oestrogen receptor status and *p53* positivity are inversely related (Cattoretti et al. 1988).

Immunohistochemically, lung tumours have been found to be positive for *p53* in 60–70% of cases (Iggo et al. 1990). The incidence of *p53*-positive cases was lower in our material with only 43% positive. This dis-

crepancy could reflect ethnic or environmental differences between *p53* expression in lung tumours in different human populations. It may partly be due to the fact that only formalin-fixed material was used in this study.

In non-small cell lung carcinomas, *p53* gene mutations have been found to be more frequent in squamous cell carcinomas than in the other histological types (Chiba et al. 1990). Similar results have also been obtained by immunohistochemistry; even small cell lung carcinomas are found to be less often *p53* positive than squamous cell carcinomas (Iggo et al. 1990). Accordingly, we also found the incidence of *p53* positivity to be higher in squamous cell carcinomas than in the other types of lung carcinomas.

Breast carcinomas have been found to be positive for the *p53* protein in 15.5% of cases (Cattoretti et al. 1988). The incidence was higher in our material and is in line with some recent immunohistochemical reports on *p53* immunoreactivity in breast carcinomas (Walker et al. 1991). Moreover, the number of *p53* mRNA transcripts is increased or absent in two-thirds of breast carcinomas (Thompson et al. 1990), suggesting that up- or down-regulated *p53* gene expression is a fairly frequent phenomenon in breast carcinoma. In our material, there were *p53*-positive cases only in ductal carcinomas. In previous reports, no association between *p53* positivity and tumour histology has been found, however (Cattoretti et al. 1988).

In the immunostaining with the *p53* antibody generally only a subpopulation of tumour cells stained positive. Consequently, there is a risk of missing some *p53*-positive cases if only one section is analysed. Because of this we studied additional sections of 11 prostate, 10 lung and 7 breast carcinomas. In 1 lung carcinoma, additional sections revealed *p53* positivity in the tumour. However 1 breast carcinoma which was *p53* positive in the first section was negative in two additional sections. Most of the primarily negative tumours (23 cases) remained negative in additional sections and all *p53*-positive tumours were positive in some (2) or all (2) of the sections. The *p53*-positive cells do not seem to be evenly distributed in the tumour tissue and there is a risk of missing a fraction of positive cases if only one section of the tumour is analysed.

Previously it has been suggested that *p53* positivity in tumours might be used as a marker of malignancy (Hall et al. 1991). This is in line with our results, since we found no positivity in benign prostate, lung or breast cells. The presence of *p53* positivity in squamous dysplasia in 1 case was associated with a squamous cell carcinoma and thus can be regarded as a part of the same process.

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