J Cancer Res Clin Oncol (1993) 119:172–178

Journal of Cancer Research and Clinical Oncology

Analysis of mutant P53 protein in osteosarcomas and other malignant and benign lesions of bone

Yoshimichi Ueda[1], Barbara Dockhorn-Dworniczak[1], Sebastian Blasius[1], Walter Mellin[1], Paul Wuisman[2], Werner Böcker[1], Albert Roessner[1]

[1] Gerhard-Domagk Institute of Pathology, University of Münster; [2] Department of Orthopaedics, University of Münster, Federal Republic of Germany

Received 26 June 1992/Accepted 14 July 1992

Summary. Alterations of tumour suppressor genes are considered crucial steps in the development of human cancers. Expressions of p53 protein, a product of the tumour suppressor gene altered most commonly in human cancers examined so far, were investigated immunohistochemically in 18 osteosarcomas and 40 other malignant and benign lesions of bone. A monoclonal antibody clone PAb240, which recognizes a common conformational epitope of mutant p53 proteins, stained nuclei of tumour cells in 12 of 18 osteosarcomas (67%). Six tumours (33%) particularly showed positive immunoreactions in more than half of the tumour cells. PAb240 also stained tumour cells in a small number of other malignant bone tumours, such as malignant fibrous histiocytoma, chondrosarcoma, and Ewing's sarcomas. Furthermore, a small number of cells of giant-cell tumours were positively stained. In contrast, PAb240 was completely negative in 21 benign bone tumours and reactive lesions examined. Another monoclonal antibody clone PAb1801, which reacts with both wild- and mutant-type p53 protein, reacted in nuclei of tumour cells of 7 osteosarcomas (39%). Most of those also reacted with PAb240. PAb1801 was expressed much more frequently in other malignant bone tumours and giant-cell tumours. In addition, PAb1801 showed intranuclear positive reactions in tumour cells of a benign chondroblastoma, and reactive cells such as actively proliferating preosteoblasts in a myositis ossificans and osteoclast-like giant cells in a giant-cell tumour. The immunoelectron-microscopic observation that p53 protein was localized in euchromatic areas of nuclei of osteosarcoma cells supported the specificity of immunoreaction for p53 protein, indicating an active role of p53 protein in the regulation of DNA synthesis and transcription. These findings suggest that point mutation of the p53 gene is frequently involved in the development of osteosarcomas. PAb240 may be a useful tool not only in screening point mutations of the p53 gene in osteosarcomas but also in the differential diagnosis between osteosarcomas and reactive bone-forming lesions. Expressions of mutant p53 protein were not correlated with any clinical or pathological factors examined, although the results should be confirmed in studies of a large number of osteosarcomas.

Key words: p53 protein – Point mutation – Osteosarcoma – Bone tumour – Immunohistochemistry – Immunoelectron microscopy

Introduction

The etiology of human osteosarcoma remains unknown. Recent studies, however, have elucidated several genetic alterations that occur during the development of osteosarcoma (Friend et al. 1986; Masuda et al. 1987; Toguchida et al. 1988; Toguchida et al. 1989; Miller et al. 1990; Araki et al. 1991). Of these, loss of alleles on chromosome 17, particularly in the defined region 17p13, is seen in more than 75% of osteosarcomas, suggesting that the alteration of a tumour suppressor gene located in this region may contribute to the development of osteosarcomas (Toguchida et al. 1989; Yamaguchi et al. 1992). p53 is a nuclear phosphoprotein that was discovered because it bound to the major transforming protein, the large T antigen of simian virus 40 (Lane and Crawford 1979; Linzer and Levine 1979). The p53 gene had been originally considered an oncogene. Recent studies, however, have clarified that the normal p53 gene actually possesses tumour suppressor activity, and that a mutant form exerts a dominant oncogenic function during in vitro transformation (Eliyasu et al. 1989; Finlay et al. 1989). The human p53 gene is located on the short arm of chromosome 17, a frequent site of allele loss in osteosarcomas (McBride et al. 1986). Since Baker et al. (1989) demonstrated a loss of both alleles, one through deletion and the other through a point mutation, in 75%–80% of colorectal cancers, point mutations of the p53 gene have been demonstrated in a number of common human cancers (Nigro et al. 1989; Takahashi et al. 1989; Chiba et al. 1990; Hollstein et al. 1991; Felix et al.

This work was supported by Deutsche Forschungs-Gemeinschaft grant Ro 648/3-1

Correspondence to: A. Roessner, Gerhard-Domagk Institute of Pathology, Münster University, Domagkstr. 17, 4400 Münster, FRG

1992; Frankel et al. 1992; Kohler et al. 1992). In the field of bone tumours, major structural alterations of the p53 gene have been shown in about 20% of osteosarcomas by Masuda et al. (1987) and Miller et al. (1990). Little is known, however, concerning point mutations of the p53 gene in osteosarcomas.

p53 protein levels are normally so low in non-transformed cells, because of the short half-life of the wild-type protein, that it cannot be detected by immunohistochemistry. In contrast, higher levels of the protein occur in a large number of tumours and tumour cell lines. It has been suggested that the increase in the concentration of the protein results from post-translational stabilization by complexing with other proteins, such as viral proteins or heat-shock proteins (Gannon et al. 1990; Rodrigues et al. 1990). Immunohistochemical techniques in studies of oncogenes and tumour suppressor genes are hampered by the problems that most antibodies cannot distinguish an overexpression of mutant forms from that of a normal form. However, a recently established monoclonal antibody PAb240, which recognizes a common conformational change of mutant forms, enables the selective detection of mutant p53 protein immunohistochemically (Gannon et al. 1990; Iggo et al. 1990).

In the present study, we immunohistochemically examine the expressions of p53 protein in 18 osteosarcomas using two different monoclonal antibodies, and compare the results with those in various malignant and benign lesions of bone. The ultrastructural distribution of p53 protein is investigated by cryo-ultramicroscopy in some osteosarcomas. The contribution of p53 gene mutation to the development of osteosarcomas, and the impact of immunohistochemistry for p53 protein on the pathological diagnosis of bone tumours are evaluated.

Materials and methods

Tumours. Eighteen osteosarcomas of bone were studied. 17 primary and 1 metastatic lesions. Most of the samples were taken by biopsy. Some samples were obtained from viable areas of the resected tumours after preoperative chemotherapy. Clinical and pathological information is briefly summarized in Table 1. Histological subtypes are defined according to Dahlin and Unni (1986), and the evaluation of response to preoperative chemotherapy recorded according to Salzer-Kuntschik et al. (1983). In addition, various benign and malignant tumours and reactive lesions of bone were examined. Malignant bone tumour comprised 7 Ewing's sarcomas, 3 chondrosarcomas, 3 malignant fibrous histiocytomas, one non-Hodgkin lymphoma, and one chordoma. Four cases of giant-cell tumours were also studied. Benign tumours and reactive lesions, a total of 21 samples, included chondroblastoma, enchondromas, osteochondromas, non-ossifying fibromas, fibrous dysplasias, eosinophilic granulomas, aneurysmal bone cysts, osteomyelitis, myositis ossificans, and callus. All of these cases had been treated from 1988 to 1991 in the Department of Orthopaedics, Münster University, and registered in the Bone Tumour Registry of Westfalia. The specimens were frozen in liquid nitrogen immediately after surgical removal, and stored at −80 C until use. For immunoelectron-microscopic examination, tumour tissues were fixed in periodate/lysine/paraformaldehyde for 2 h at 4° C. After washing in phosphate-buffered saline (PBS), tissue cubes of 0.5×0.5 mm were transformed to a solution of 1.15 M sucrose in PBS at 4 C, followed by 2.3 *M* sucrose in PBS. After saturation, the specimens were mounted on aluminium carriers, and rapidly frozen in liquid nitrogen. Tissue immediately adjacent to that stored was fixed in 4% buffered formalin for histopathological diagnosis. DNA analysis of osteosarcomas was based on microspectrometry. Sections (10 µm) from paraffin-embedded specimens were deparaffinized, and staining of cell nuclei was performed by a Feulgen procedure. The DNA measurements were carried out in a static image analysis system (Aurens ACAS cytometry analysis system). Twenty normal lymphocytes and 200 tumour cells were analysed for each tumour specimen. The median DNA value of each control cell population was given the arbitrary DNA index value of 1.0, denoting the diploid DNA content, and values of discrete peaks in each tumour specimen were measured.

Immunohistochemistry. Two monoclonal antibodies to p53 protein were applied: clone PAb240 (Oncogene Science Inc., Manhasset, N.Y.), a mouse monoclonal antibody that does not react to wild-type p53 protein but recognizes a common conformational epitope of the mutant type (Gannon et al. 1990); clone PAb1801 (Novocastra Laboratories, England), a mouse monoclonal antibody specific to human p53, either wild-or mutant-type (Banks et al. 1986). All tumours were also stained with Ki-67 (Dakopatts, Glostrup, Denmark), a mouse monoclonal antibody against a cell proliferation antigen, to evaluate the viability of tumour cells. All antibodies were detected by the alkaline phosphatase/anti-(alkaline phosphatase) (APAAP) method (Cordell et al. 1984).

Table 1. Osteosarcomas examined[a]

Case	Age (years)/sex	Location of primary tumour	Histological subtype	Response to preop. chem.	DNA index
1	17 / M	Dist. femur	Anaplastic	V	NE
2	24 / M	Dist. femur	Fibroblastic	II	NE
3	21 / F	Prox. humerus	Telangiectatic	V	1.0; 2.2
4	20 / M	Prox. tibia	Fibroblastic	IV	NE
5	15 / M	Prox. femur	Fibroblastic	I	1.3
6	28 / M	Prox. tibia	Osteoblastic	II	1.0; 1.5
7	20 / M	Pelvis	Chondroblastic	IV	1.5
8			Telangiectatic		NE
9	11 / M	Dist. femur	Osteoblastic	II	NE
10	19 / M	Dist. femur	Osteoblastic	III	1.8
11	59 / F	Prox. humerus	Anaplastic	V	NE
12	17 / M	Dist. femur	Osteoblastic	II	1.8
13	11 / F	Prox. femur	Chondroblastic	V	NE
14	14 / F	Dist. femur	Chondroblastic	III	NE
15	12 / M	Dist. femur	Osteoblastic	IV	1.5
16	27 / M	Pelvis	Chondroblastic	IV	1.4; 2.0
17	23 / F	Prox. humerus	Osteoblastic	IV	NE
18	48 / M	Pelvis	Osteoblastic	V	1.0

[a] Preop. chem., preoperative chemotherapy; Prox., proximal; Dist., distal; NE, not examined; I, II, III, IV, V, VI, Salzer-Kuntschik's classification

Briefly, 6-μm cryostat sections, air-dried for 1 h, were fixed in acetone at –20 C for 10 min for PAb1801, and for 30 s for Ki-67. Sections for PAb240 were not fixed. After being rinsed in TRIS-buffered saline (145 m*M* NaCl, 20 m*M* TRIS, pH 7.6), the sections were exposed to primary antibodies for 1 h at room temperature. The working dilutions of PAb240, PAb1801 and Ki-67, which were decided after careful titration on appropriate targets, were 1:400, 1:400, 1:80 respectively. After washing with TRIS-buffered saline, rabbit antibody against mouse immunoglobulins (Dekopatts) was applied for 30 min, and APAAP complex (Dakopatts) for 45 min. Colour development was performed with naphthol AS-BI phosphate and new fuchsin for 25 min. For positive control, staining on a cryostat section of breast cancer that showed a positive reaction both with PAb240 and PAb1801 was used on every run. For negative control, a monoclonal antibody to cytokeratin, clone KI-1 (Dianova, Hamburg, FRG), was applied as the primary antibody, or the primary antibody was omitted. Immunoreactions were scored as follows: +++, more than 50% of tumour cells are positive; ++, 10%–50% of tumour cells are positive; +, less than 10% of tumour cells are positive; ±, positive only in a few tumour cells; –, tumour cells are negative.

Immunoelectron microscopy. Ultra-thin sections were preincubated with 0.1 M phosphate buffer including 0.2 *M* NaCl, 1% bovine serum albumin, and 5% normal goat serum. The sections were then incubated with the monoclonal antibody clone PAb1801 at a concentration of 1000 ng/ml for 1 h, and by goat anti-(mouse IgG) conjugated with 10 nm colloidal gold (Auro-Probe GAM 10 nm; Janssen Life Science, Belgium) at a dilution of 1:50 for 1 h. For negative control, primary antibody was omitted. After the immunolabelling, the sections were fixed in 2% glutaraldehyde for 10 min, contrasted with 2% uranyl acetate oxalate and uranyl acetate, and finally stabilized by a 1.1% tylose membrane. The sections were observed with a Phillips 400 electron microscope.

Results

The monoclonal antibody clone PAb240 showed granular positive immunoreactions in nuclei of osteosarcoma cells. Nucleolar regions were sometimes distinctly spared (Fig. 1A). The proportion and distribution of positive cells in osteosarcoma tissues were variable from tumour to tumour and even with in the same tumour. Some variation was also observed in the intensity of staining. Nuclei of non-neoplastic cells in osteosarcoma tissues were completely negative. Osteosarcomas sometimes showed weak intracytoplasmic reactions with PAb240. We did not consider those reactions as specific, since cytoplasm of normal osteoblasts was also stained weakly with the same antibody in a few cases. Out of 18 osteosarcomas studied, 12 (67%) showed positive cells with PAb240. Most of them presented positive reactions in more than 10% of tumour cells. Intense and diffuse positive reactions, in more than 50% of tumour cells were seen in 6 cases (33%). PAb1801 also exhibited an intranuclear granular positive reaction in osteosarcoma cells (Fig. 1B). No significant positive reactions were detected in non-neoplastic cells in the osteosarcoma tissue. Out of 18 osteosarcomas, 7 (39%) presented positive immunoreactions with PAb1801. Most of these were also positive with PAb240. Staining intensities of PAb1801 were comparable to those of PAb240 in most tumours (Table 2). No correlation could be seen between the expressions of p53 protein and clinical features, such as the age of the patients and their response to preoperative chemotherapy, or pathological features including the location of tumours or histological subtypes. DNA ploidies were measured in 9 osteosarcomas. The other tumour specimens were not suitable for DNA morphometry because of the strong de-

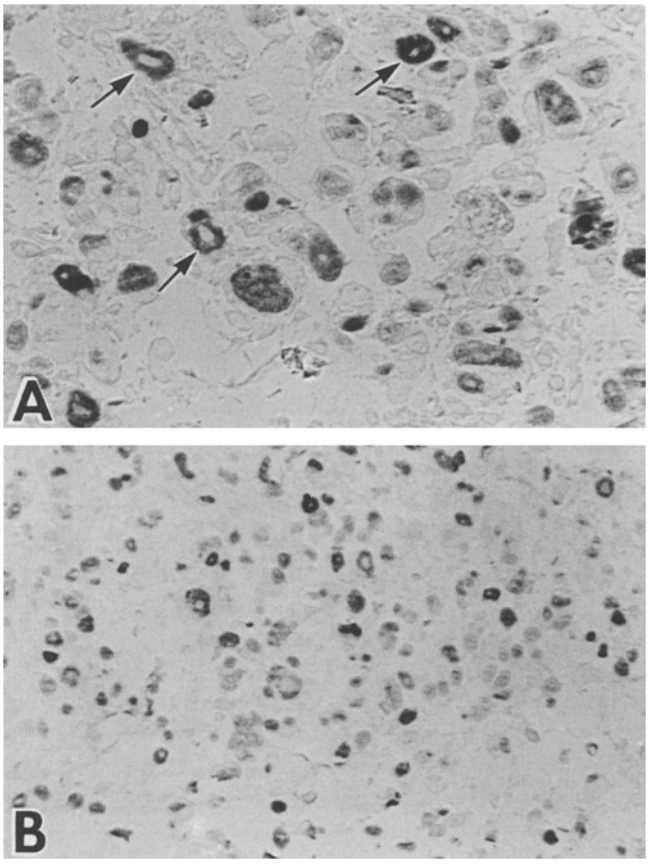

Fig. 1A,B. Immunostaining of osteosarcoma (case 11) with monoclonal antibody clone PAb240 (**A**) and clone PAb1801 (**B**). **A** Nuclei of more than 50% of tumour cells show granular positive immunoreactions of various levels of intensity. Nucleolar regions are distinctly spared in some positive tumour cells (*arrows*). **B** Clone PAb1801 also reacts with nuclei of tumour cell with various intensities. No nuclear staining with haematoxylin. **A** ×520, **B** ×375

Table 2. Expressions of p53 protein in osteosarcomas

Case	Immunoreactions[a] with	
	PAb240	PAb1801
1	+++	–
2	+++	+++
3	++	+++
4	–	–
5	+++	+
6	–	–
7	–	–
8	–	–
9	+++	–
10	+++	–
11	+++	+++
12	+	–
13	±	+
14	++	–
15	++	–
16	++	+++
17	–	–
18	++	++

[a] +++, more than 50% of tumour cells are positive; ++, 10%–50% of tumour cells are positive; +, fewer than 10% of tumour cells are positive; ±, positive only in a few tumour cells; –, tumour cells are negative

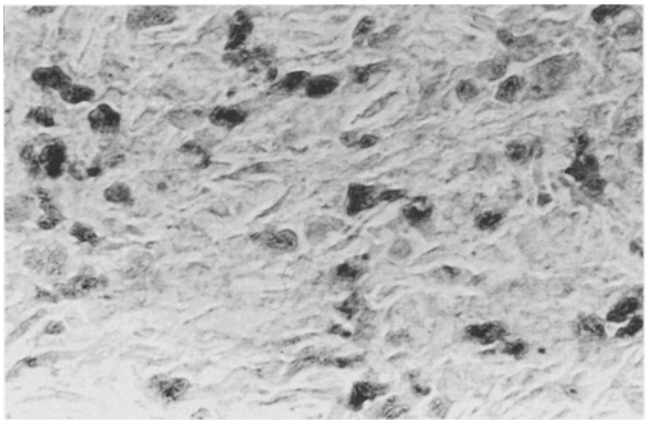

Fig. 2. Immunostaining of malignant fibrous histiocytoma with PAb1801. About 50% of tumour cells show intranuclear positive reactions. No nuclear staining with haematoxylin. ×520

Fig. 4. Immunostaining of myositis ossificans with PAb1801. A single preosteoblast actively proliferating around woven bone (*WB*) shows a distinct positive reaction in the nucleus (*arrow*). Nu nuclear staining with haematoxylin. ×375

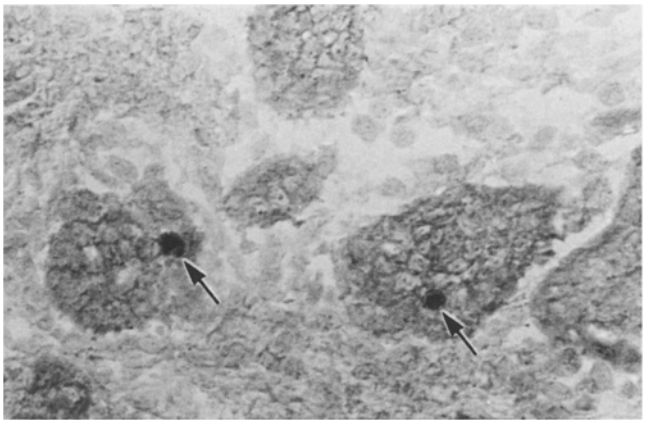

Fig. 3. Immunostaining of giant-cell tumour with PAb1801. PAb1801 is expressed in the nuclei of some osteoclast-like multinucleated giant cells (*arrows*). In this picture, stromal cells show no positive reactions. No nuclear staining with haematoxylin. ×520

Table 3. Expressions of p53 protein in osteosarcomas and other bone tumours and reactive lesions

Lesion	Number of cases examined	Number of cases positive with	
		PAb240	PAb1801
Osteosarcoma	18	12	7
Chondrosarcoma	3	1	1
Malignant fibrous histiocytoma	3	1	3
Ewing's sarcoma	7	2	6
Non-Hodgkin lymphoma	1	0	0
Chordoma	1	0	1
Giant-cell tumour	4	2	2
Benign tumour and reactive lesions	21	0	2[a]

[a] Chondroblastoma and myositis ossificans

calcification. One tumour was diploid and 8 tumours showed aneuploid stemlines. In 2 of those 8 osteosarcomas, an additional diploid tumour stemline coexisted with the aneuploid one. No correlation was seen between the expression of p53 protein and DNA ploidy patterns of osteosarcoma (Table 1).

PAb240-positive cells were also seen in a small number of malignant bone tumours other than osteosarcoma: 2 of 7 Ewing's sarcomas, 1 of 3 chondrosarcomas, and 1 of 3 malignant fibrous histiocytomas. The numbers of PAb240-positive cells were small except in 1 malignant fibrous histiocytoma. In Ewing's sarcomas particularly, the numbers of PAb240-positive cells were small, fewer than 1% of tumour cells, and staining intensities were weak. In contrast, malignant bone tumours other than osteosarcoma frequently and intensely expressed positive reactions with PAb1801:6/7 Ewing's sarcomas, 1/3 chondrosarcomas, 3/3 malignant fibrous histicytomas (Fig.2), and 1/1 chordoma. PAb1801 stained all tumours that were positive with PAb240 (Table 3). A small number of stromal cells, fewer than 1%, showed positive reactions with PAbv240 in 2 of 4 giant-cell tumours. PAb1801 was also demonstrated in those cases, and PAb1801-positive cells were much more numerous than PAb240-positive ones. In addition, PAb1801 was expressed in nuclei of some osteoclast-like multinucleated giant cells of a giant-cell tumour (Fig.3).

None of the benign bone lesions reacted with PAb240. In contrast, PAb1801 was expressed in tumour cells of 1 of 2 chrondroblastomas and in actively proliferating preosteoblasts in 1 case of myositis ossificans (Fig.4).

Immunoelectron-microscopic examinations were performed on 2 osteosarcomas: on examination with PAb1801, p53 protein was mainly localized in the euchromatic ares of nuclei in osteosarcoma cells. Heterochromatic areas, nucleoli, nuclear membrane, and cytoplasm were hardly labelled for p53 protein (Fig. 5A). No significant deposition of gold particles was seen in the negative control (Fig. 5B). The immunoelectron-microscopic trial with PAb240 was not successful.

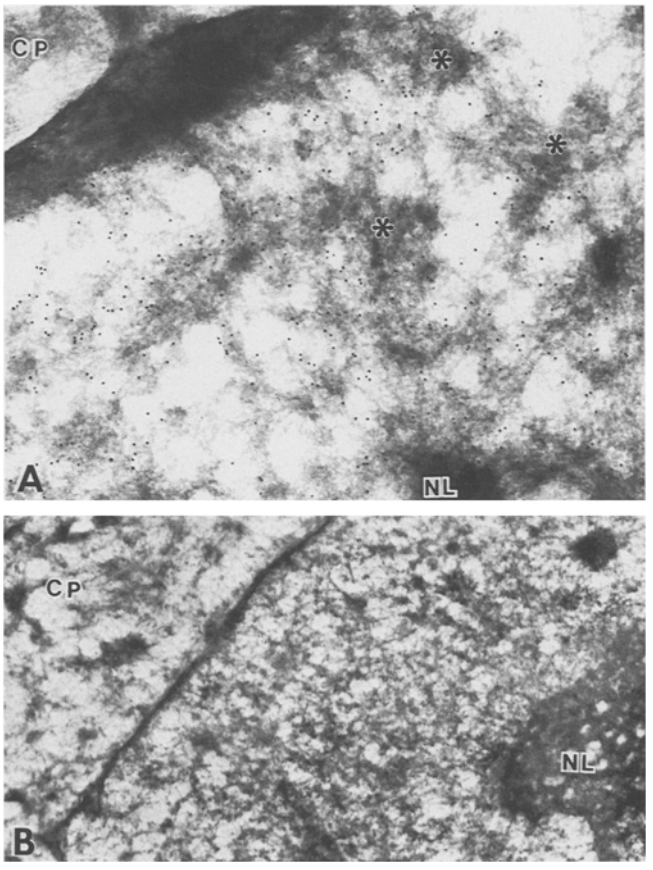

Fig. 5A, B. Immunoelectron micrographs of osteosarcoma (case 11) with PAb1801 (**A**) and negative control (**B**). **A** Euchromatic areas of tumour nucleus are intensely labelled by PAb1801. Heterochromatic area (*), nucleolus (*NL*), nuclear membrane, and cytoplasm (*Cp*) are negative for labelling. **B** Negative control shows no significant labelling of the tumour cells with PAb1801. **A** ×25 000, **B** ×12 000

Discussion

Missense point mutations in the p53 gene are the most frequently identified genetic change in human cancers (Nigro et al. 1989; Takahashi et al. 1989; Chiba et al. 1990; Hollstein et al. 1991; Felix et al. 1992; Frankel et al. 1992; Kohler et al. 1992). Gannon et al. (1990) recently established a monoclonal antibody to p53 protein, designated PAb240, which does not react with wild-type p53 protein but recognizes mutant forms of p53 protein resulting from various activating mutations. This suggests that the different point mutations exert a common conformational effect on the protein. The presence of the common conformational change is also supported by the evidence that mutant p53 protein fails to bind to simian virus 40 (SV40) large T antigen and associates with heat-shock protein, while wild-type p53 protein binds to SV40 large T antigen but not to heat-shock protein (Gannon et al. 1990). The current immunohistochemical study using this monoclonal antibody showed that mutant p53 protein is frequently (67%) expressed in the nuclei of osteosarcoma cells. In 33% of the osteosarcomas, particularly positive reactions were seen in more than half of the tumour cells. These frequent and widespread expressions of mutant p53 protein in osteosarcomas suggest that mutation of p53 gene is one of the crucial steps in the development of osteosarcomas.

PAb240 was positive also in a small number of other malignant bone tumours such as malignant fibrous histiocytoma, chondrosarcoma, and Ewing's sarcomas. Furthermore, cells in giant-cell tumours reacted with PAb240. Although giant-cell tumours usually show benign biological behaviour, they possess a metastatic potential in approximately 2% of cases and change to a highly malignant form in 4% of cases (Ladanyi et al. 1989). In contrast, mutant p53 was completely negative in the benign bone tumours and reactive lesions examined. These findings are consistent with the previous immunohistochemical studies on expressions of mutant p53 protein in colorectal tumours and lung cancers (Iggo et al. 1990; Purdie et al. 1991). Iggo et al. (1990) showed the mutant p53 protein in 70% of lung cancers, but in no normal lung tissue. They confirmed the existence of a mutation of the p53 gene in representative cases. Purdie et al. (1991) demonstrated the appearance of mutant p53 protein not only in about 50% of colorectal carcinomas but also in some adenomas belonging to the more dysplastic categories, in which the risk of transition to invasive carcinoma is higher, suggesting a strong relationship between mutant p53 expression and malignant transformation in colorectal tumours. The current findings in various tumours and reactive lesions of bone further support the concept that mutant p53 arises relatively late in the neoplastic progression, and may correlate with increasing dysplasia, malignant transformation and increased tumour aggressiveness.

In the current literature, the discussion continues concerning p53 as a possible tumour marker (Cossman and Schlegl 1991; Hall et al. 1991; Wynford-Thomas 1991). The extensive study of Porter et al. (1992) using PAb1801 has shown that in a series of 255 tumours positive immunostaining was noted in a wide variety of malignant tumours, but not in benign tumours or in non-neoplastic tissues. Our immunohistochemical results with the antibody PAb1801, however, seem to limit this suggestion, since the antibody presented positive reactions in some benign tumours and reactive lesions of bone. On the other hand, the result with the antibody PAb240 did not reveal any positive reaction in benign bone tumours or reactive bone lesions. Therefore, the selection of the antibody might be of importance for the utility of p53 expression as a possible marker of malignant tumours in bone tumour pathology. If more extensive studies confirm these data, it will be the first time that reactive bone-forming lesions, such as myositis ossificans or aneurysmal bone cysts, can be discerned from highly malignant osteosarcoma not only by skilled examination of conventional tissue stains, but also by biological characteristics of the osteosarcoma cells.

The expression of mutant p53 protein was not correlated with any clinicopathological factors examined. Response to preoperative chemotherapy is considered a good parameter for favourable prognosis in osteosarcoma (Rosen et al. 1982). Recently Look et al. (1990) and Mankin et al. (1991) reported that the presence of diploid tumour stemlines resulted in a poorer prognosis is than did aneuploid osteosarcoma. In the present study, 7 of 17 osteosarcomas were responders to preoperative chemotherapy. As to DNA ploidy, one osteosarcoma showed an entirely diploid stemline, and an additional diploid tumour stemline coexisted with aneuploid stemlines in two other osteosarcomas. Expressions of mutant p53 protein did not correlate with responsiveness to

preoperative chemotherapy, or with the pattern of DNA ploidy of the osteosarcomas. Therefore, expression of p53 protein does not seem to be related to the prognosis of osteosarcoma. However, the clinical significance of expression of mutant p53 protein should be decided in a future study of a large number of cases with complete follow-up.

The exact function of p53 protein in normal cells is still unclear. Recent studies suggested that p53 protein controls the cell cycle by negatively regulating gene transcription, perhaps a set of genes that effect the passage from G_1 to S phase (Levine et al. 1991). The present immunoelectron-microscopic study using the antibody PAb1801 clearly localized p53 protein in the euchromatic area of nuclei of osteosarcoma cells, in which DNA is actively synthesized and transcribed (Bloom and Fawcett 1975). Our findings indicate morphologically the contribution of p53 protein to the regulation of DNA synthesis and/or transcription. Furthermore, the characteristic localization of p53 protein further supports the specificity of the present immunohistochemical findings.

Recently, several monoclonal antibodies have been established against p53 protein (Walker et al. 1991). Comparative studies using those antibodies reported that PAb1801 and PAb240 stained in a similar pattern and frequency in common human cancers (Mark et al. 1991; Purdie et al. 1991; Walker et al. 1991). Compared with those results, the present findings in osteosarcomas must be considered unexpected. Of the 12 osteosarcomas reacting positively with PAb240, 6 did not react with PAb1801. The impact of technical differences seems to be less plausible, because PAb1801 frequently and intensely stained malignant bone tumours other than osteosarcomas. Mutant p53 proteins seem to be heterogeneous, structurally and functionally (Levine et al. 1991). Mutant p53 protein expressed in osteosarcomas may have a different conformation from that in other common cancers. The exact nature of mutant p53 protein in osteosarcoma should be further investigated on the biochemical and biophysical, as well as on the DNA level.

References

Araki N, Uchida A, Kimura T, Yoshikawa H, Aoki Y, Ueda T, Takai S, Miki T, Ono K (1990) Involvement of the retinoblastoma gene in primary osteosarcomas and other bone and soft tissue tumors. Clin Orthop 270:271–277

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

Banks L, Matlashewski G, Crawford L (1986) Isolation of human p53 specific monoclonal antibodies and their use in the studies of human p53 expression. Eur J Biochem 159:529–534

Bloom W, Fawcett DW (1975) A text book of histology, 10th edn. Saunders, Philadelphia

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

Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz M, MacDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatases (APAAP) complexes. J Histochem Cytochem 32:219–229

Cossman J, Schlegel R (1991) p53 in the diagnosis of human neoplasia. JNCI 83:980–981

Dahlin DC, Unni KK (1986) Bone tumours. General aspects and data on 8542 cases, 4th edn. Thomas, Springfield Ill

Eliyasu D, Michalovitz D, Eliyasu S, Pinhasi-Kimhi O, Oren M (1989) Wild-type p53 can inhibit oncogene-mediated focus formation. Proc Natl Acad Sci USA 86:8763–8767

Felix CA, Kappel CC, Mitsudomi T, Nau MM, Tsokos M, Crouch GD, Nisen PD, Winick NJ, Helman LJ (1992) Frequency and diversity of p53 mutations in childhood rabdomyosarcoma. Cancer Res 52:2243–2247

Finlay CA, Hinds PW, Levine AJ (1989) The p53 proto-oncogene can act as a suppressor of transformation. Cell 57:1083–1093

Frankel RH, Bayona W, Koslow M, Newcomb EW (1992) p53 mutations in human malignant gliomas: comparison of loss of heterozygosity with mutation frequency. Cancer Res 52:1427–1433

Friend SH, Bernard R, Rogeli S, Weinberg RA, Rapaport JM, Albert DM, Drija TP (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 323:643–646

Gannon JV, Greaves R, Iggo R, Lane P (1990) Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for mutation for the mutant form. EMBO J 9:1595–1602

Hall PA, Ray A, Lemoine NR, Midgley CA, Kransz T, Lane DP (1991) p53 immunostaining as a marker of malignant disease in diagnostic cytopathology. Lancet 338:513–514

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

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

Kohler MF, Berchuck A, Davidoff AM, Humphrey PA, Dodge RK, Igelhart JD, Soper JT, Clarke-Pearson DL, Bast RC, Marks JR (1992) Overexpression and mutation of p53 in endometrial carcinoma. Cancer Res 52:1622–1627

Ladanyi M, Traganos F, Huvos AG (1989) Benign metastasizing giant cell tumors of bone. A DNA flow cytometric study. Cancer 64:1521–1526

Lane DP, Crawford LV (1979) T antigen is bound to a host protein in SV40-transformed cells. Nature 278:261–263

Levine AJ, Momand J, Finlay CA (1991) The p53 tumour suppressor gene. Nature 351:453–456

Linzer DIH, Levine AJ (1979) Characterization of a 54K dalton cellular SV40 tumour antigen present in SV40 transformed cells and uninfected embryonal carcinoma cells. Cell 17:43–52

Look AT, Douglass EC, Meyer WH (1988) Clinical importance of near-diploid tumor stem lines in patients with osteosarcoma of an extremity. N Engl J Med 318:1567–1572

Mankin HJ, Gebhardt MC, Springfield DS, Litwak GJ, Kusazaki K, Rosenberg AE (1991) Flow cytometric studies of human osteosarcoma. Clin Orthop 270:169–180

Marks JR, Davidoff AM, Humphrey PA, Pence JC, Dodge RK, Clarke-Pearson DL, Iglehart JD, Bast RC, Berchuck A (1991) Overexpression and mutation of p53 in epithelial ovarian cancer. Cancer Res 51:2979–2984

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

McBride OW, Merry D, Givol D (1986) The gene for human p53 cellular tumor antigen is located on chromosome 17 short arm (17p13). Proc Natl Acad Sci USA 83:130–134

Miller CW, Aslo A, Tsay C, Slamon D, Ishizaki K, Toguchida J, Yamamuro T, Lampkin B, Koeffler HP (1990) Frequency and structure of p53 rearrangements in human osteosarcoma. Cancer Res 50:7950–7954

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

Porter PL, Gown AM, Kramp SG, Coltrera MD (1992) Widespread p53 overexpression in human malignant tumors. An immunohistochemical study using methacarn-fixed, embedded tissue. Am J Pathol 140:145–153

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

Rodrigues NR, Rowan A, Smith MEF, Kerr IB, Bodmer WF, Gannon JV, Lane DP (1990) p53 mutations in colorectal cancer. Proc Natl Acad Sci USA 87:7555–7559

Rosen G, Caparros B, Huvos AG, Kosloff C, Nirenberg A, Cacavio A, Marcove RC, Lane JM, Mehta B, Urban C (1982) Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. Cancer 49:1221–1230

Salzer-Kuntschik M, Delling D, Beron G, Sigmund R (1983) Morphological grades of regression on osteosarcoma after polychemotherapy – Study COSS 80. J Cancer Res Clin Oncol 106:21–24

Takahashi T, Nau MM, Chiba I, Birrer MJ, Rosenberg RK, Vinocour M, Levitt M, Pass H, Gazdar AF, Minna JD (1989) p53: a frequent target for genetic abnormalities in lung cancer. Science 246:491–494

Toguchida J, Ishizaki K, Sasaki MS, Ikenaga M, Sugimoto M, Kotoura Y, Yamamuro T (1988) Chromosomal reorganization for the expression of recessive mutatioin of retinoblastoma susceptibility gene in the development of osteosarcoma. Cancer Res 48:3939–3943

Toguchida J, Ishizaki K, Nakamura Y, Sasaki MS, Ikenaga M, Kato M, Sugimoto M, Kotoura Y, Yamamuro T (1989) Assignment of common allele loss of osteosarcoma to the subregion 17p13. Cancer Res 49:6247–6251

Walker RA, Dearing SJ, Lane DP, Varley JM (1991) Expression of p53 protein in infiltrating and in situ breast carcinomas. J Pathol 165:203–211

Wynford-Thomas D (1991) Oncogenes and anti-oncogenes; the molecular basis of tumour behaviour. J Pathol 165:187–201

Wynford-Thomas D (1992) p53 in tumour pathology: can we trust immunocytochemistry? J Pathol 166:329–330

Yamaguchi T, Toguchida J, Yamamuro T, Kotoura Y, Takada N, Kawaguchi N, Kaneko Y, Nakamura Y, Sasaki MS (1992) Allelotype analysis in osteosarcoma – frequent allele loss on 3q, 17p, and 18q. Cancer Res 52:2419–2423