Virchows Arch (1995) 426:563–569

ORIGINAL ARTICLE

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Elevated content of p53 protein in the absence of p53 gene mutations as a possible prognostic marker for human renal cell tumors

Received: 29 November 1994 / Accepted: 11 April 1995

Abstract p53 tumour suppressor gene expression was estimated immunohistochemically using DO-1 monoclonal antibody (recognising both wild-type and mutant p53) in 88 human renal tumours. Single strand conformation polymorphism (SSCP) analysis of possible mutations within exons 4–8 of the p53 gene was performed in 29 of the tumours (mostly immunostaining-positive cases). Obviously elevated p53 content was detected with DO-1 antibody in chromophobic cell carcinomas and most clear/chromophilic cell tumours (in chromophilic cell populations). In contrast, clear cell carcinomas demonstrated either complete absence of p53 expression or the presence of single immunopositive nuclei. Oncocytomas were completely negative. Additional immunostaining of the positive samples with mutant p53-specific Pab240 monoclonal antibody failed to detect immunopositive material. No p53 mutation was found in any of the samples analysed by SSCP. Our results suggest that the elevated p53 content in human renal cell carcinomas does not result from gene mutation and that p53 gene alterations are probably not an important mechanism in the development of human renal cell carcinomas. Accumulation of the wild-type p53 protein may be a useful prognostic marker indicating neoplastic progression and malignancy.

Key words Kidney tumours · Tumour suppressor gene · Immunohistochemistry · SSCP

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Introduction

Renal cell carcinomas (RCCs) are the most frequent malignant tumours of the human kidney. Studies on the pathobiology of renal carcinogenesis in animal models have revealed that this process is much more diverse than was anticipated some years ago [4]. In addition to the proximal nephron, the collecting duct system has been established as a frequent site of origin of renal cell tumours in both laboratory animals and humans [4, 43]. In accordance with the changing concept of the histogenesis of renal cell tumours, several entities demonstrating characteristic differences in cellular phenotype have been distinguished. This has prompted Thoenes et al. [45] to propose a new classification of human renal cell tumours, which is based mainly on cytological criteria and distinguishes the following types: (i) clear cell carcinoma; (ii) chromophilic cell carcinoma; (iii) chromophobic cell carcinoma; (iv) oncocytoma; (v) Bellini duct carcinoma. Although this classification is demonstrably of clinical relevance, precise evaluation of the prognosis for RCC of different cytological types remains complicated, and there is an active search for additional diagnostic approaches, such as use of various immunohistochemical, cytogenetic and molecular markers [19, 21, 25, 34, 36, 42, 47, 49].

It is generally accepted that alterations of the structure and/or expression of the p53 tumour suppressor gene are closely related to the process of tumour growth. The gene is believed to play an important role in the control of cell proliferation, so that loss of its normal function may lead to uncontrolled cell proliferation such as is characteristic for neoplastic progression [9, 24, 32, 50]. Numerous reports describe p53 mutations in diverse human tumours (see [16] for review). The mutant forms of p53 protein are known to be significantly more stable than wild-type protein molecules and this property of the protein offers an excellent opportunity to use its immunohistochemical detection as a diagnostic test. Expression of p53 has been studied immunohistochemically in a number of neoplasms [6, 17, 27, 39, 46, 48] where a

good correlation between p53 protein expression and tumour stage has been shown. These reports comprise observations obtained in colorectal, head and neck, lung, urinary bladder, gastric, oesophageal and ovarian cancers [18, 26, 35, 41, 48, 52].

We investigated both p53 protein expression and mutations of the corresponding gene in a collection of human kidney tumours (predominantly RCC), searching for possible correlations of immunohistochemical and molecular changes with cell type and other pathomorphological variables in the tumours. This approach seemed to be attractive in terms of developing new diagnostic and prognostic criteria for this complex type of malignancy. Moreover, we expected to obtain valuable new information on the role of p53 in human renal cell tumour development.

Both wild-type and mutant p53 proteins have been demonstrated on paraffin sections and in positive cases immunostaining was repeated using frozen material. Parallel frozen sections were immunostained with an additional antibody recognising mutant p53 exclusively. Selected tumour areas (usually demonstrating elevated content of p53 protein) were dissected from serial tissue sections and subjected to single strand conformation polymorphism (SSCP) analysis to reveal possible p53 mutations. This combined approach provided a comprehensive estimate of correlations of tumour histopathology, p53 protein content, and corresponding gene alterations.

Materials and methods

Eighty-eight kidney tumour specimens obtained from patients undergoing unilateral nephrectomy (at the Department of Urology, Surgical Centre University of Heidelberg) were used for the analysis. All the tissue samples were either immediately fixed with Carnoy mixture (and eventually embedded in paraffin) or frozen in isopentane at –150° C and stored at –80° C. Serial sections were prepared from each tissue block. Haematoxylin and eosin (HE) staining and periodic acid-Schiff (PAS) reaction were employed for diagnostic histopathology. During histopathological analysis of HE-stained sections nuclear grade was assigned according to the least differentiated area of tumour using existing criteria [12, 14].

Immunohistochemical detection of p53 protein was carried out using two anti-p53 antibodies: DO-1 (Dianova, Germany) recognising both mutant and wild types of the protein; and Pab 240 (Dianova), which is designed to detect only mutant forms of the p53 [13] and can be used only with frozen material.

Paraffin sections were deparaffinized and heated in the microwave oven as previously described [40]. After washing in 0.15 M TBS the sections were preincubated with normal goat serum for 30 min at room temperature, then incubated with primary antibody DO-1 (dilution 1:200) for 18 h at 4° C. Secondary biotinylated goat anti-mouse IgG antibody (Bio SPA; dilution 1:200) was applied for 30 min at room temperature, followed by incubation with streptavidin-biotinylated alkaline phosphatase complex (dilution 1:200, BioSPA). Fast Red was used as the final chromogen, and sections were counterstained with Mayer's haemalaum.

Frozen sections were fixed in 7% formalin for 10 min at room temperature and further subjected to the treatment described above. Avidin-biotin blocking was used prior to application of the secondary antibody.

Human hepatocellular tumour samples previously shown to bear p53 mutations [22] were used as positive controls. Additional control reactions were performed in the absence of the anti-p53 antibodies.

The slides were evaluated by two independent observers for the presence and proportion of positively stained cells. p53 staining was scored as: 1 (–) negative; 2 (+) weakly positive (presence of single positive cells); 3 (++) positive (numerous positive cells and occasional positive cell clusters); 4 (+++) strongly positive (multiple clusters of strongly positive cells).

Twenty-nine tumours were analysed for the presence of p53 mutations. For this purpose, serial cryostat sections were prepared from each specimen. Some of these sections were mounted on dialysis membranes. For the identification of tumour tissue HE staining, PAS reaction, and immunostaining with the DO-1 antibody were employed. Selected areas of tumour tissue (usually demonstrating elevated p53 expression level) were dissected from the sections with a scalpel blade together with supporting dialysis membrane. PCR amplification of exons 4–8 of the p53 gene was performed as described earlier [22]. The following amplification primers were used:

Exon 4 HD1: 5′ – AAG CAA TGG ATG ATT TGA TGC TG – 3′
HD11: 5′ – TGG TAG GTT TTC TGG GAA GGG AC – 3′
Exon 5 HA1: 5′ – CCT CTT CCT GCA GTA CTC CCC TG – 3′
HC11: 5′ – AAG ATG CTG AGG AGG GGC GAG AC – 3′
Exon 6 HE1: 5′ – ACC ATG AGC GCT GCT CAG ATA GC – 3′
HE11: 5′ – AGT TGC AAA CCA GAC CTC AGG CG – 3′
Exon 7 HB1: 5′ – GTT GGC TCT GAC TGT ACC ACC AT – 3′
HB11: 5′ – GCT CCT GAC CTG GAG TCT – 3′
Exon 8 HA2: 5′ – CTA TCC TGA GTA GTG GTA ATC TA – 3′
HA11: 5′ – GCT TGC TTA CCT CGC TTA GTG CT – 3′

PCR products were subjected to SSCP analysis [33] according to a previously published procedure [22]. In some cases of p53 exon 7 analysis (questionable SSCP results), initial PCR products were sequenced following purification with QIAquickSpin purification columns (Quiagen, Chatsworth, Calif.). Samples were sequenced in both directions using 5′-^{32}P-labelled HB1 and HB11 as sequencing primers. DNA sequencing was performed with TAQuence cycle-sequencing kit (US Biochemicals, Cleveland, Ohio).

Results

Initially all 88 renal tumours studied were subdivided histopathologically according to the prevailing cell type. Among 85 renal cell tumours there were 33 clear RCCs, 47 clear/chromophilic RCCs, 2 chromophobic RCCs, and 3 oncocytomas. Three non-epithelial tumours comprised one spindle-cell sarcoma and two fibrosarcomas. The results of both immunohistochemical and molecular analyses are summarised in Table 1.

In general, immunostaining of paraffin sections with DO-1 antibody showed a positive reaction mostly in the nuclei, but sometimes minor cytoplasmic staining was detected. In mitotic tumour cells, reactions were usually weaker than in interphase nuclei.

There was an evident difference in the immunoreactivity between different types of RCC. Clear cell carcinomas are predominantly composed of clear cells, but often contain a variable proportion of more acidophilic (granular) cells. The nuclear grade of the tumours was 1–2. As a rule, the PAS reaction demonstrated the presence of significant amounts of glycogen in the tumour cells. p53 expression was not detectable in most of these cases. In only in 7 cases were scattered single cells showing positive immunostaining seen (Fig. 1a).

Clear/chromophilic cell carcinomas consisted of clear and acidophilic (granular) cells with nuclear grade 2–3.

Table 1 Results of immunohistochemical detection of p53 protein and single-strand conformation polymorphism (SSCP) mutation analysis of the corresponding gene in human renal tumours (*RCC* renal cell carcinoma)

Tumour type	Number of tumours	Nuclear grade	p53-immunostaining-positive tumours and distribution of cases according to the positive reaction intensity	p53 mutation analysis (SSCP-positive/tested)
Clear-cell RCC	33	1–2	7 (21%) 7+	0/3
Clear/chromophilic RCC	47	2–3	34 (72%) 12+ 16++ 6+++	0/24
Chromophobic RCC	2	2–4	2 (100%) 1++ 1+++	–
Oncocytoma	3	1–2	0	–
Fibrosarcoma	2	2	2 (100%) 2+++	0/2
Spindle-cell sarcoma	1	1	0	–

Sometimes cells with slight basophilia were observed. The cytoplasm of acidophilic and basophilic cells was usually poor in glycogen. p53 immunoreactivity was found in 34 of 47 clear/chromophilic cell tumours. It must be emphasised that p53-positive nuclei were observed predominantly in acidophilic areas or in those composed of intermediate cells (Fig. 1b). The number of positive cells and the staining intensity varied dramatically, both between carcinomas and within individual tumours. This heterogeneity meant it was difficult to compare expression levels of p53 in different tumours. There was no clear correlation between nuclear grade and immunoreactivity. Moreover, it is noteworthy that as a rule clusters of p53-positive cells gave the impression of a kind of clonality.

Chromophobic cell tumours are a relatively rare, but distinct subtype of human RCCs. These tumours are composed of cells with a finely reticular and light cytoplasm (the glycogen content is low, as demonstrated by the PAS reaction, but there is strong binding of colloidal iron, suggesting an accumulation of glycosaminoglycans). Only two tumours of this type were analysed, and both of them displayed strongly positive immunostaining for p53 (Fig. 1c).

Oncocytomas are composed of large cells with a finely granular and strongly acidophilic cytoplasm. We failed to detect any expression of p53 in three immunostained oncocytomas.

The three non-epithelial tumours examined in this study comprised one spindle-cell sarcoma (p53-negative) and two fibrosarcomas (strongly positive for p53 immunostaining).

In most cases, immunostained sections contained normal kidney tissue adjacent to the tumours. These areas were always negative except in a few cases of clear/chromophilic and chromophobic cell tumours. In the latter cases single positive cells were seen in tubular epithelium.

As described in the previous section, two different antibodies were used in attempts to detect p53 protein immunohistochemically. Immunostaining with DO-1 antibody recognising both wild-type and mutant p53 protein was initially performed on paraffin sections and regarded as successful. To confirm these results (described above; see also Table 1), all positive cases were reanalysed using frozen material with both DO-1 antibody and Pab-240 antibody recognising a common conformational epitope of mutant forms of p53 protein resulting from different p53 gene mutations [13]. While all the tumours used in these additional test were positive with DO-1, we failed to obtain even a single positive result with Pab-240 antibody.

After completion of the immunohistochemical part of the study, 29 tumour samples were selected for analysis of possible p53 gene alterations. Most of the samples tested were shown to be obviously positive for p53 immunostaining. SSCP analysis of the amplified fragment of p53 gene corresponding to exons 4, 5, 6, 7, and 8 was carefully performed. An SSCP banding pattern typical for this study is presented in Fig. 2. All the samples gave negative SSCP results during analysis of exons 4, 5, 6, and 8. Initial analysis of exon 7 revealed several samples with slight mobility shifts (data not shown), but repeated SSCP procedure followed by double-sided sequencing allowed us to confirm the absence of any detectable amounts of mutant p53 in these cases.

Discussion

The development of renal cell tumours is associated with characteristic phenotypic cellular changes. At the same time, some cytogenetic and molecular genetic alterations are typical of these neoplasms. Reports of a high proportion of 3p deletions in both early and advanced renal cell tumours [3, 20, 53] have led to the suggestion that the loss of a tumour suppressor gene located on chromosome 3p may be an early (initiating) event in the history of these tumours. Several other chromosomal abnormalities are described [36, 49], but observations of loss of heterozygosity on chromosome 17p [29, 30, 37] are of particular interest, since p53 tumour suppressor gene is local-

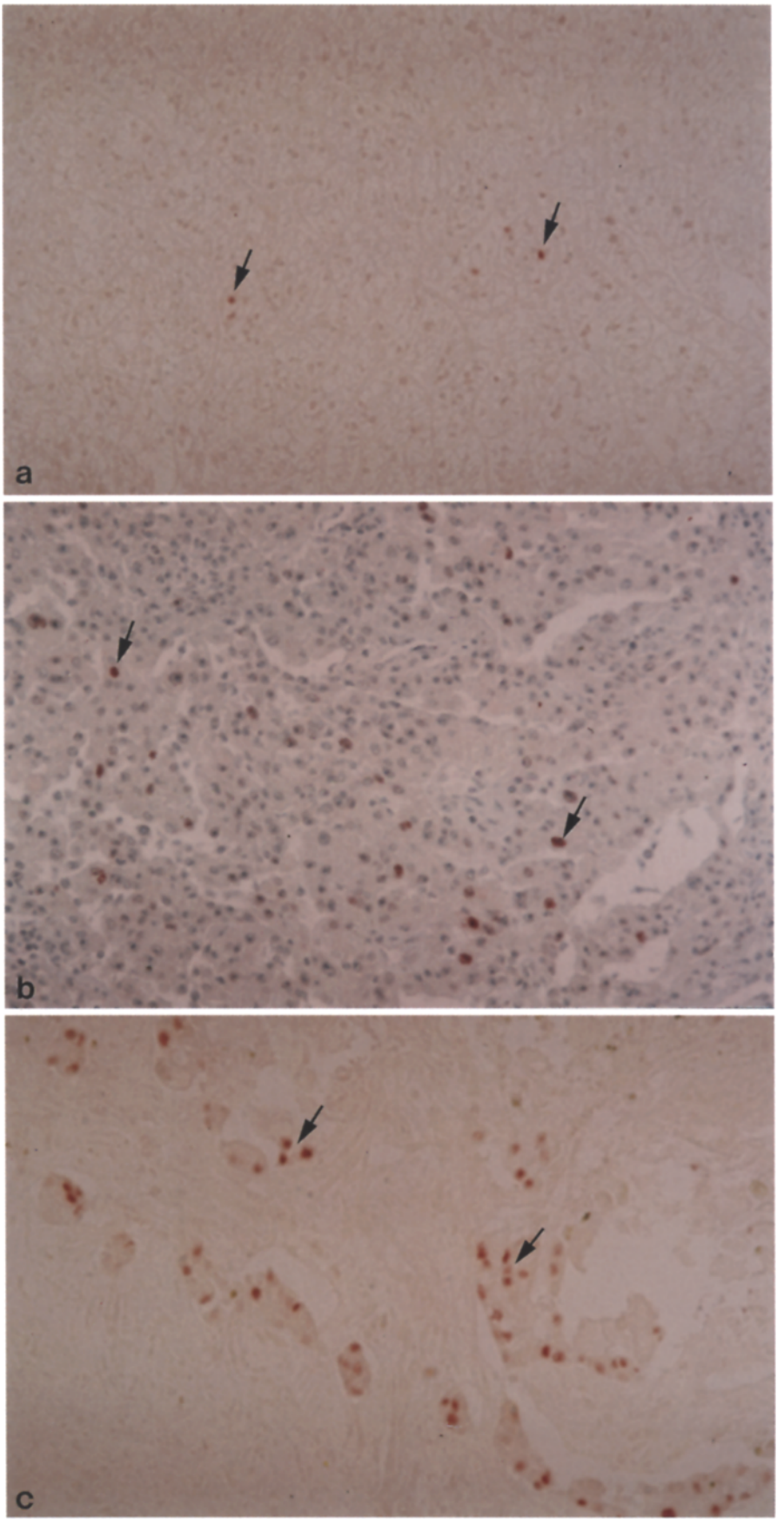

Fig. 1a–c Immunostaining for p53 with DO-1 antibody: **a** clear cell carcinoma, ×310; **b** chromophilic cell carcinoma, ×310; **c** chromophobic cell carcinoma, ×310. *Arrows,* p53-positive nuclei

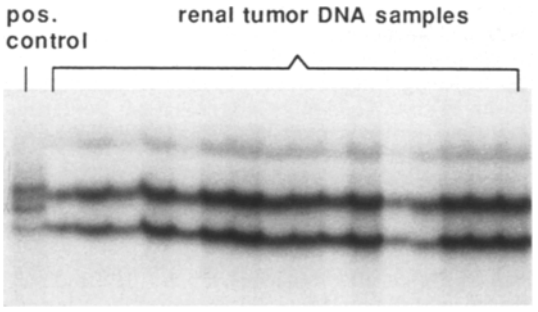

Fig. 2 A typical negative result of the single-strand conformation polymorphism (SSCP) analysis (p53 exon 5). A DNA sample from human hepatocellular carcinoma bearing A to G transition at the second position of codon 163 was used as a positive control. The sample was generously provided by Dr. S. Kress

ised on the 17p. It seemed to be logical to imply that p53 alterations may be of importance for RCC progression, and several groups of investigators have addressed this problem. Suzuki et al. [44] reported that evaluation of RCC samples using the PCR-SSCP strategy allowed detection of only one point mutation of p53 in 23 RCCs. Similar results (2 mutation-positive of 118 tested RCCs) have been obtained recently by another group [19]. However, Reiter et al. [37] observed a high proportion (33%) of cases positive for p53 mutation when analysing cell lines produced from RCCs; however, it may be argued that in this case clones of cells possessing p53 mutations developed only under cell culture conditions, as was demonstrated in cell lines established from mouse liver tumours [23].

The present study has combined cellular phenotyping and immunohistochemical detection of p53 gene product with the analysis of mutations in the highly conserved region of the gene. The results show that an elevated content of p53 is quite frequent in human RCCs, especially in components of clear/chromophilic RCCs and in chromophobic cell tumours. It is important to stress that there was a clear positive correlation between accumulation of p53 protein and the histopathological patterns reflecting relative aggressivity of the tumours [1, 4, 5, 43]. Prognostically favourable oncocytomas and clear cell tumours were mostly p53-negative, whereas more aggressive clear/chromophilic and chromphobic cell tumours frequently displayed p53 immunoreactivity. Furthermore, within tumours containing areas of clear and chromophilic cells only chromophilic (acidophilic or granular) areas shown to be more advanced towards malignancy [1, 5, 8, 43] displayed p53-positive immunostaining. These clear-cut findings allow us to exclude any possibility of false-positive immunostaining, which is known to be a common danger in immunohistochemical detection of p53 [15]. At the same time, we failed to detect any positive case when applying the PCR-SSCP strategy for p53 mutation analysis. These data may be interpreted as evidence of mutation-independent accumulation of p53. In this connection, the difference between the immunostaining results obtained with two different antibodies further corroborates this conclusion. Pab-240 monoclonal antibody recognises an epitope located between amino acids 156 and 214 of p53. This epitope is believed to be expressed as the result of a common conformational effect exerted by different p53 mutations [13]. We were unable to detect any positive reaction using Pab240, whereas the same samples showed distinctly positive immunostaining with DO-1 antibody. The finding is easy to explain by mere absence of the mutant p53 in any of our samples. Similar results demonstrating immunohistochemical detection of wild-type p53 protein have been reported recently for colorectal adenomas [46], bone and soft tissue sarcomas [51], astrocytomas [38], and glioblastomas [2].

Another important question arising from our results is related to the causes of elevated p53 product in a significant number of the tumours tested. While it is believed that in most cases the rise of p53 protein results from increased stability brought about by p53 gene mutation [10], alternative mechanisms may be suggested. First, it is difficult to exclude the possibility that in some cases the p53 overproduction (probably due to alteration of some other regulatory gene/s) is so massive that unstable wild-type protein becomes detectable by immunostaining. Similarly, the protein may be a subject of post-translational modification or can perhaps be stabilised with other cellular proteins. The latter possibility, which now seems to be the more likely [11], has been demonstrated for the *mdm2* oncogene product p90, which is able to form a complex with the wild-type p53 protein [7, 8, 28]. It is interesting to note that *mdm2* amplification has been reported in about 30% of human sarcomas [31], especially in view of our observation of extremely intense p53 immunostaining (in the absence of p53 gene mutations) in two cases of fibrosarcoma (see Table 1). Other, still unknown, mechanisms may also be involved in the accumulation of wild-type p53. Finally, some uncommon mutations outside exons 4–8 of p53 might be responsible for the accumulation of p53 in some cases; however, such an explanation is highly improbable because mutations of this kind are so rare.

The results of this study allow us to conclude that p53 mutation is not important in the progression of human RCCs. This conclusion supports the data obtained by other investigators in different human populations [19, 44]. However, post-translational alterations of the structure and/or function of the wild-type p53 protein seem to be important especially for chromophilic and chromophobic cell tumours. Augmentation of immunohistochemically detectable p53 protein content may be proposed as a prognostic test, indicating increased aggressivity. This conclusion is further supported by recent data published by Uhlman et al. [47], demonstrating an association of positive p53 immunostaining in RCCs with acquisition of metastatic potential.

Acknowledgements We are indebted to Professor G. Staehler (Department of Urology, Surgical Centre, University of Heidelberg) for providing the tumour samples. We thank Dr. S. Kress for his useful suggestions on the SSCP analysis. We also thank Erika Filsinger and Gabriele Schmidt for excellent technical assistance.

References

1. Ahn YS, Zerban H, Grobholz R, Bannasch P (1992) Sequential changes in glycogen content, expression of glucose transporters and enzymic patterns during development of clear/acidophilic cell tumors in rat kidney. Carcinogenesis 13:2329–2334
2. Ali IU, Schweitzer JB, Ikejiri B, Saxena A, Robertson JT, Oldfield EH (1994) Heterogeneity of subcellular localization of p53 protein in human glioblastomas. Cancer Res 54:1–5
3. Anglard P, Trahan E, Liu S, Latif F, Merino MJ, Lerman MI, Zbar B, Linehan WM (1992) Molecular and cellular characterization of human renal cell carcinoma cell lines. Cancer Res 52:348–356
4. Bannasch P, Zerban H (1990) Animal models and renal carcinogenesis. In: Eble JN (ed) Tumors and tumor-like conditions of the kidneys and ureters. Churchill Livingstone, New York, pp 1–34
5. Baretton G, Kuhlmann B, Krech R, Löhrs U (1991) Intratumoural heterogeneity of nuclear DNA-content and proliferation in clear cell type carcinomas of the kidney. Virchows Arch [B] 61:57–63
6. Bennett WP, Hollstein MC, He A, Zhu SM, Resau JH, Trump BF, Metcalf RA, Welsh JA, Midgley C, Lane DP, Harris CC (1991) Archival analysis of p53 genetic and protein alterations in Chinese esophageal cancer. Oncogene 6:1779–1784
7. Chen C-Y, Oliner JD, Zhan Q, Fornace AJ, Vogelstein B, Kastan MB (1994) Interactions between p53 and MDM2 in a mammalian cell cycle checkpoint pathway. Proc Natl Acad Sci USA 91:2684–2688
8. Delahunt B, Nacey JN (1987) Renal cell carcinoma. II. Histological indicators of prognosis. Pathology 19:258–263
9. Donehower LA, Bradley A (1993) The tumor suppressor p53. Biochim Biophys Acta 1155:181–205
10. Finlay CA, Hinds PW, Tan T-H, Eliyahu D, Oren M, Levine AJ (1988) Activating mutations for transformation by p53 produce a gene product that forms an hcs70-p53 complex with an altered half-life. Mol Cell Biol 8:531–539
11. Fritsche M, Haessler C, Brandner G (1993) Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. Oncogene 8:307–318
12. Fuhrman SA, Lasky LC, Limas C (1982) Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 6:655–663
13. Gannon JV, Greaves R, Iggo R, Lane DP (1990) Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. EMBO J 9:1595–1602
14. Gelb AB, Shibuya RB, Weiss LM, Medeiros LJ (1993) Stage I renal cell carcinoma: a clinicopathologic study of 82 cases. Am J Surg Pathol 17:275–286
15. Hall PA, Lane DG (1994) p53 in tumor pathology: can we trust immunohistochemistry? J Pathol (Lond) 172:1–4
16. Hollstein M, Sidransky D, Vogelstein B, Harris CC (1991) p53 mutations in human cancers. Science 253:49–53
17. Iggo R, Gatter K, Bartek J, Lane D, Harris AL (1990) Increased expression of mutant form of p53 oncogene in primary lung cancer. Lancet 335:675–679
18. Imai S, Kiyozuka Y, Nishimura H, Iwanaga S, Murakami F, Imamura K, Noda T, Haga S, Yakushiji M (1994) Overexpression of the tumor suppressor gene p53 in human ovarian tumor tissues and its correlation with clinical stage. Int J Oncol 4: 1097–1103
19. Kikuchi Y, Kishi T, Suzuki M, Furusato M, Aizawa S (1994) Polymerase chain reaction – single strand conformation polymorphism analysis of the p53 gene in paraffin-embedded surgical material from human renal cell carcinomas. Virchows Arch 424:229–233
20. Kovacs G (1993) Molecular differential pathology of renal cell tumors. Histopathology 22:1–8
21. Kovacs A, Störkel S, Thoenes W, Kovacs G (1992) Mitochondrial and chromosomal DNA alterations in human chromophobe renal cell carcinomas. J Pathol (Lond) 167:273–277
22. Kress S, Jahn U-R, Buchmann A, Bannasch P, Schwarz M (1992) p53 mutations in human hepatocellular carcinomas from Germany. Cancer Res 52:3220–3223
23. Kress S, König J, Schweizer J, Löhrke H, Bauer-Hoffman R, Schwarz M (1992) p53 mutations are absent from carcinogen-induced mouse liver tumors but occur in cell lines established from these tumors. Mol Carcinog 6:148–158
24. Levine AJ, Perry ME, Chang A, Silver A, Dittmer D, Wu M, Welsh D (1994) The 1993 Walter Hubert Lecture: The role of the p53 tumour-suppressor gene in tumorigenesis. Br J Cancer 69:409–416
25. Makos M, Nelkin BD, Reiter ER, Gnarra JR, Brooks J, Isaaks W, Linehan M, Baylin SB (1993) Regional DNA hypermethylation at D17S5 precedes 17p structural changes in the progression of renal tumors. Cancer Res 53:2719–2722
26. Maruyama K, Shiozaki H, Shimaya K, Inoue M, Iwazawa T, Matsui S, Mori T (1994) p53 expression in esophageal dysplasia: a possible biomarker for carcinogenesis of esophageal squamous cell carcinoma. Int J Oncol 4:1061–1065
27. Mayall FG, Goddard H, Gibbs AR (1992) p53 immunostaining in the distinction between benign and malignant mesothelial proliferation using formalin-fixed paraffin sections. J Pathol (Lond) 168:377-381
28. Momand J, Zambetti GP, Olson DC, George D, Levine AJ (1992) The *mdm2* oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 69: 1237–1245
29. Ogawa O, Habuchi T, Kakehi Y, Koshiba M, Sugiyama T, Yoshida O (1992) Allelic losses of chromosome 17p in human renal cell carcinoma are inversely related to allelic losses at chromosome 3p. Cancer Res 52:1881–1885
30. Oka K, Ishikawa J, Bruner JM, Takahashi R, Saya H (1991) Detection of loss of heterozygosity in the p53 gene in renal cell carcinoma and bladder cancer using the polymerase chain reaction. Mol Carcinog 4:10–13
31. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B (1992) Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 358:80–83
32. Oren M (1992) p53: the ultimate tumor suppressor gene? FASEB J 6:3169–3176
33. Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879
34. Ortmann M, Vierbuchen M, Koller G, Fischer R (1988) Renal oncocytoma. I. Cytochrome C oxidase in normal and neoplastic renal tissue as detected by immunohistochemistry – a valuable aid to distinguish oncocytoma from renal cell carcinoma. Virchows Arch [B] 56:165–173
35. Pavelic ZP, Gluckman JL, Gapany M, Reising J, Craven JM, Kelley D, Pavelich L, Gapany S, Biddinger P, Stambrook PJ (1992) Improved immunohistochemical detection of p53 protein in paraffin-embedded tissues reveals elevated levels in most head and neck and lung carcinomas: correlation with clinicopathological parameters. Anticancer Res 12:1389–1394
36. Presti JC, Rao PH, Chen Q, Reuter VE, Li FP, Fair WR, Jhanwar SC (1991) Histopathological, cytogenetic, and molecular characterization of renal cortical tumors. Cancer Res 51: 1544–1552
37. Reiter RE, Anglard P, Liu S, Gnarra JR, Linehan WM (1993) Chromosome 17p deletions and p53 mutations in renal cell carcinoma. Cancer Res 53:3092–3097
38. Rubio M-P, Deimling A von, Yandell DW, Wiestler OD, Gusella JF, Louis DN (1993) Accumulation of wild type p53 protein in human astrocytomas. Cancer Res 53:3465–3467
39. Schneider J, Rubio MP, Rodriguez-Escudero FJ, Seizinger BR, Castresana JS (1994) Identification of p53 mutations by means of single strand conformation polymorphism analysis in gynaecological tumours: comparison with the results of immunohistochemistry. Eur J Cancer 30A:504–508
40. Shi SR, Key ME, Kalra KL (1991) Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 39:741–748

41. Soini Y, Turpeeniemi-Hujanen T, Kamel D, Autio-Harmainen H, Risteli J, Risteli L, Nuorva K, Pääkkö P, Vähäkangas K (1993) p53 immunohistochemistry in transitional cell carcinoma and dysplasia of the urinary bladder correlates with disease progression. Br J Cancer 68:1029–1035
42. Steinberg P, Störkel S, Oesch F, Thoenes W (1992) Carbohydrate metabolism in human renal clear cell carcinomas. Lab Invest 67:506–511
43. Störkel S (1993) Karzinome und Onkozytome der Niere. Fischer, Stuttgart
44. Suzuki Y, Tamura G, Satodate R, Fujioka T (1993) Infrequent mutation of p53 gene in human renal cell carcinoma detected by polymerase chain reaction – single stranded conformation polymorphism analysis. Jpn J Cancer Res 83:233–235
45. Thoenes W, Störkel S, Rumpelt HJ (1986) Histopathology and classification of renal cell tumors (adenomas, oncocytomas and carcinomas). The basic cytological and histopathological elements and their use for diagnostics. Pathol Res Pract 181: 125–143
46. Tominaga O, Hamelin R, Trouvat V, Salmon RJ, Lesec G, Thomas G, Remvikos Y (1993) Frequently elevated content of immunochemically defined wild-type p53 protein in colorectal adenomas. Oncogene 8:2653–2658
47. Uhlman DL, Nguyen PL, Manivel JC, Aeppli D, Resnick JM, Fraley EE, Zhang G, Niehans GA (1994) Association of immunohistochemical staining for p53 with metastatic progression and poor survival in patients with renal cell carcinoma. J Natl Cancer Inst 86:1470–1475
48. Van den Berg FM, Tigges AJ, Schipper MEI, den Hartog-Jager FCA, Kroes WGM, Walboomers JMM (1989) Expression of the nuclear oncogene p53 in colon tumors. J Pathol (Lond) 157:193–199
49. Van den Berg E, Van der Hout AH, Oosterhuis JW, Störkel S, Dijkhuizen T, Dam A, Zweers HMM, Mensink HJA, Buys CHCM, de Jong B (1993) Cytogenetic analysis of epithelial renal-cell tumors: relationship with a new histopathological classification. Int J Cancer 55:223–227
50. Vogelstein B, Kinzler KW (1992) p53 finction and dysfunction. Cell 70:523–526
51. Wadayama B, Toguchida J, Yamaguchi T, Sasaki MS, Kotoura Y, Yamamuro T (1993) p53 expression and its relationship to DNA alterations in bone and soft tissue sarcomas. Br J Cancer 68:1134–1139
52. Yonemura Y, Fushida S, Tsugawa K, Ninomiya I, Fonseca L, Yamaguchi A, Miyazaki I, Urano T, Shiku H (1993) Correlation of p53 expression and proliferative activity in gastric cancer. Anal Cell Pathol 5:277–288
53. Zbar B, Brauch H, Talmadge C, Linehan M (1987) Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature 327:721–727