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REVIEW ARTICLE

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The clinical significance of p53 aberrations in human tumours

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Abstract p53 aberrations are the most common genetic alteration found in human tumours and this review summarizes the current understanding of the clinical significance of p53 abnormalities. Immunohistochemical and molecular techniques can demonstrate alterations at the protein and gene level, respectively, but with a significant discordance between the findings of either technique. The tumours evaluated in this review include cancers of the breast, lung, gastrointestinal tract, genitourinary tract, and others. In most cases, only data on p53 protein are available and in each of these tumour types discrepant conclusions on the clinical value of p53 abnormalities as prognostic indicators have been reached. The role of p53 in the context of anticancer adjuvant therapy has also been analysed. Experimental data suggest that p53 affects the apoptotic response to anticancer agents, but this has not yet been proven in a clinical series where this demonstration and its effect on therapy could represent one of the most important endpoints in p53 clinical research. The use of standardized techniques to evaluate p53 gene mutation and protein accumulation within controlled clinical series of patients entering prospective trials is essential to answer the many remaining questions on the clinical significance of p53 aberrations.

Key words p53 · Immunohistochemistry · Human tumours

The p53 gene: general introduction

In the 15 years following the discovery of the tumour suppressor gene p53, a tremendous amount of basic and applied research has been directed to dissecting the physiological functions of p53 protein, and aberrations of the p53 gene have been linked to many different types of human malignancies. p53 mutations represent the single most common genetic alteration in human tumours.

This nuclear phosphoprotein was initially discovered as an oligomeric complex with the large T antigen in SV40-transformed cells [83, 88]. Since the large T antigen is essential to maintain the transformed phenotype in these cells, p53 was originally classified as a tumour antigen. Subsequently, a number of p53 DNA clones were isolated and found capable of immortalizing cells [71] and of cooperating with the *ras* oncogene in transforming cells in culture [35, 116]. At this point p53 was therefore considered a dominant oncogene. In 1989, however, all the transforming p53 clones were revealed to harbour p53 mutations [61] and, in addition, it was demonstrated that wild-type p53 was capable of suppressing cell transformation by other oncogenes [42]. Thus p53 became established as a tumour suppressor gene: since then it has been shown that p53 deletions and/or point mutations are common in a wide spectrum of human tumours [113].

Several comprehensive reviews have described the current status of our understanding of p53 function and dysfunction (in the neoplastic process) [52, 56, 63, 86, 87, 152]. The purpose of this review is to focus on the clinical implications of p53 aberrations in common human solid tumours. By this we mean that we will consider p53 alterations as they may directly relate to patient care. At the present time the gene product may influence clinical oncology in three ways: (1) as a possible unfavourable prognostic indicator; (2) as a possible predictor of tumour chemo and/or radioresistance; and (3) as a target for gene therapy. The latter is the focus of extensive research for future applications [45] and will not be examined further in this review. We will therefore limit our survey to p53 as a prognostic variable and as a possible indicator of increased tumour resistance to conventional adjuvant therapy. This will be preceded by a short commentary on the techniques commonly used to detect p53 aberrations in solid tumours.

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Detection of p53 aberrations: immunohistochemistry and molecular techniques

Loss of function of p53 protein may result from gene mutations leading to the synthesis of ineffective, mutated/truncated proteins or to the complete lack of p53 protein synthesis, as would be the case in bi-allelic gene deletions. Alternatively, loss of function may be due to inactivation of wild-type p53 protein by its untimely/unregulated degradation (as mediated by the E6 protein of HPV16/18) [78, 84] or by binding to cellular proteins, like mdm-2 [104, 118]. Accordingly, studies aimed at assessing the role of p53 loss of function in different pathological conditions may require the evaluation of both p53 gene and protein status.

However, one of the most exciting facets of p53 aberrations is that the vast majority of gene mutations (missense mutations clustered in exons 5 through 8) code for mutated proteins with prolonged half-life (up to several hours), which accumulate within the cell nuclei and can be detected immunohistochemically [10]. This is at variance with wild-type p53 protein, which has a very short half-life (6–30 min), and is not normally detectable by immunohistochemical techniques (see below for ever increasing exceptions to this long-held dogma). Accordingly, the immunohistochemical detection of p53 accumulation has been considered to mirror the occurrence of missense gene mutations.

Immunohistochemical staining techniques suitable for documenting p53 immunoreactivity in formalin-fixed and paraffin-embedded tissue sections – after pretreatment with proteolytic enzymes or with deionized water or citrate buffer in microwave ovens – have recently been refined [2, 37, 76, 101]. This has allowed retrospective investigations on large series of cases and the exploration of the clinical usefulness of p53 aberrations. Immunocytochemical investigations currently represent the most convenient means of assessment of the role of p53 abnormalities in tumour development and progression.

Some intrinsic limitations and controversial aspects of the immunohistochemical approach, however, have raised concerns about the reliability and reproducibility of the findings:

1. Most of the available antibodies to p53 protein recognize both wild-type and mutated proteins, not being specific for the latter. The demonstration of intracellular p53 accumulation cannot be taken as definite evidence of gene mutation, because it could be due either to an upregulated expression of the wild-type gene, or to the binding of normal p53 to a variety of cellular proteins. Studies aimed at assessing the correlation between immunoreactivity and gene mutations have reported a higher degree of correlation for PAb 1801 and DO7 monoclonal antibodies than for the polyclonal antiserum CM1 [4, 16].
2. Gene abnormalities other than missense mutations in exons 5 through 8 do not lead to the accumulation of aberrant proteins, and thus escape immunohistochemical detection. Also, loss of function of p53 protein by viral-induced degradation cannot be documented immunohistochemically.
3. The different antibodies to p53 have different specificities and sensitivities, so that the immunohistochemical findings are – at least in part – dependent on the choice of the antibody. Studies on the same tumour types may provide contrasting findings when diverse antibodies to p53 are used [4, 82, 143].
4. The type and length of fixation and of pretreatment of the tissue sections, as well as the working concentration of the primary antibodies and the choice of the detection procedure, may affect the final result significantly. The correspondence between immunoreactivity in fresh frozen tissues and their fixed and embedded counterparts has been reported to vary from 83% to 100%, with no "false-positive" results in fixed and embedded tissues [22, 76]. In our experience, the working dilution of the primary antibodies is particularly critical, especially with regard to the PAb1801 monoclonal antibody. This antibody is most effective at very high dilutions of the commercially available preparations (1:1000–1:1400). When used at lower dilutions, it will unexpectedly immunostain a significantly lower number of cells, possibly due to competitive interactions between the antibody molecules. Interestingly, reports documenting lower effectiveness of this monoclonal antibody (mAb) in comparison with other mAbs to p53 all used the PAb 1801 at very high concentration (1:10–1:20) [82, 143].
5. The percentage of p53-immunoreactive neoplastic cells is often highly variable among different tumours of the same histotype, and in diverse fields of the same tumour. In some investigations, cases have been considered to be immunoreactive irrespective of the number of stained cells, whereas in other studies different cut-off values (more commonly staining of at least 10% of the neoplastic cells) have been used to discriminate immunoreactive from non-immunoreactive cases [6]. This is an additional bias which makes it even more difficult to correlate the results of different investigations.
6. Cytoplasmic accumulation of p53 protein has been considered variably: in some studies it has been disregarded as non-specific, whereas in others it has been taken into account, turning out to be a more powerful prognostic indicator than nuclear accumulation [14, 140].
7. p53 immunoreactivity is not restricted to neoplastic (or dysplastic) cells. With the currently available antibodies a minor subset of normal cells shows nuclear p53 immunoreactivity in a variety of human tissues [1, 12, 30]. This is most commonly seen in the basal cell layers of stratified epithelia [1, 12]. Recently, a mAb to p53 protein has been reported to immunostain (in frozen tissue sections) the majority of normal resting lymphocytes consistently, and the terminally differentiated epithelial cells of the prostate, gastrointestinal and respiratory tract. Interestingly, immunoreactivity in these normal cells is restricted to the cytoplasm and the perinuclear areas [117]. Furthermore, wild-type p53 accumulates in cells exposed to genotoxic stress [138], indicating that

p53 is involved in the nucleotide excision repair pathway.

The assessment of gene mutations in human tumours has been greatly improved by the use of polymerase chain reaction (PCR)-based techniques; the approach to detection of mutations, however, depends on several characteristics of the gene to be studied and on the material to be analysed [92].

p53 gene mutations can be demonstrated by DNA sequencing and analysis of DNA mobility shifts. Direct sequencing of single- or double-stranded PCR amplified DNA (or reverse transcribed RNA) can identify the type of mutation and its precise location. Several recent innovations, including the use of magnetic beads for DNA strand separation and purification, direct sequencing of double-stranded DNA with PCR, and the use of non-radioisotopic techniques, have made DNA sequencing more easily applicable and have significantly improved its reliability. Direct sequencing, however, can identify p53 mutations only when wild-type alleles do not exceed two-thirds of the material to be analysed [25]. Therefore, since clinical tumour specimens may frequently contain a large amount of stromal and inflammatory cells, and not all tumour cells may harbour p53 gene mutations, direct sequencing may produce false-negative results. Additionally, all types of DNA sequencing techniques are expensive because they are labour intensive and/or require sophisticated equipment. These limitations have made the use of sequence analysis uncommon as a screening technique to detect p53 mutations in human tumours. DNA sequencing, however, remains the technique of choice to characterize the mutations found by mobility shift analysis.

The most commonly used techniques to detect DNA mobility shifts are denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and single-strand conformation polymorphism (SSCP). DGGE and TGGE rely on the principle that DNA fragments different even by a single nucleotide will show different melting behaviour under denaturing conditions [110, 132, 154]. The denaturant is a chemical substance in DGGE (formamide, urea), whereas a physical denaturant (temperature) is used in TGGE. SSCP analysis relies on the premise that even a single base substitution in a relatively short fragment of DNA (<300 bases) will determine a modification in the secondary structure of denatured (single-strand) DNA [59, 114]. As a consequence, the DNA fragments will migrate with different speed in non-denaturing polyacrylamide gels. The amplification of the DNA fragment of interest by PCR greatly facilitates the techniques and improves sensitivitiy.

These techniques have been successfully employed to detect point mutations of many different genes in a variety of tissues with diverse experimental conditions. In particular, p53 mutations have been demonstrated with a satisfactory sensitivity by both DGGE and SSCP analysis [60, 107, 108]. Although the SSCP technique originally included radioisotopic labelling of PCR fragments, it can be successfully performed using non-radioactive substances (silver stains and fluorochromes) to visualize DNA frgments. The sensitivity of the non-radioactive techniques may approach that obtained with radioisotopic SSCP [16, 32, 37, 133].

To date, as described in detail in the following sections, the vast majority of investigations that have focused on the prognostic significance of p53 aberrations have used immunohistochemical techniques. Therefore most studies evaluated "p53 protein accumulation", without any correlation with the gene status.

The relative weight of this bias favouring immunohistochemistry (IHC) can be fully understood by considering the data reviewed by Greenblatt et al. [52]; in 84 studies in which both ICH and sequence analysis were performed on the same tumours, 44% of cases showed p53 immunoreactivity, whereas only 36% displayed p53 gene mutations. The sensitivity of IHC for the identification of gene mutations in the reviewed studies was 75% (range 36–100%) and the positive predictive value was only 63% (range 8–100%). For this reason p53 accumulation cannot be automatically equated to p53 gene mutations. Consequently, the prognostic significance of p53 accumulation may not necessarily parallel that of p53 mutations. Furthermore, accumulation of p53 protein, in the absence of p53 gene mutations, may actually represent a "gain of function", which might contribute to tumorigenesis by mechanisms that remain to be fully elucidated.

Clinical implications of p53 aberrations in human tumours

The following sections summarize the data available in the literature in commonly occurring human solid tumours. The studies that are referenced in the tables accompanying each section are not referenced in the text. Considering the fact that an extremely large number of papers on p53 have appeared in the last few years (in excess of 2500 from 1990 only) we limit our review to studies addressing its prognostic significance on series of patients with clinical follow-up. Studies limited to correlative investigations with more established prognostic parameters have been excluded, except when they provided particularly significant information. Whenever possible or relevant, we have added further data from the reviewed studies, such as the additional therapy after surgery, and particular (or unusual) characteristics of the patient populations or of the results obtained.

Breast cancer

Breast carcinomas have been the most extensively investigated neoplasms. The studies available in the literature to date are summarized in Table 1. Seven investigations evaluated breast cancer patients without lymph node me-

Table 1 Breast carcinomas (*FU* follow-up, *DFS* disease-free survival, *OS* overall survival, *IHC* immunohistochemistry, *DGGE* denaturing gradient gel electrophoresis, *SSCP* single strand conformation polymorphism, *NA* not available, *NS* not significant, *NN* node-negative, *NP* node-positive

First author [ref. no.]	Method	Number positive (%)	FU (mean)	DFS/OS
Node negative				
Isola [67]	IHC	42/289 (14%)	8.5 years	NA/0.001
Bosari [11]	IHC	32/124 (25%)	10 years	NS/NS
Allred [2]	IHC/SSCP	362/700 (52%)	54 months	0.05–0.0001/0.21–0.003
Silvestrini [137]	IHC	113/256 (44%)	72 months	0.0001/0.0001
Elledge [36]	SSCP	28/200 (14%)	71 months	0.01/NA
Gasparini [51]	IHC	57/203 (28%)	62 months	0.004/0.02
Rosen [123]	IHC	95/440 (21%)	10 years	NS/NS
Mixed				
Ostrowski [115]	IHC	32/90 (36%)	28 months	NA/NS
Iwaya [69]	IHC	NN: 8/35 (23%) NP: 9/38 (24%)	>50 months	NA/0.01
Thor [145]	IHC	NN: 29/131 (22%) NP: 32/128 (25%)	7 years	0.018/0.057 0.008/0.0005
Sawan [129]	IHC	63/197 (32%)	<36 months	NN: NA/NS NP: NA/NS
Poller [119]	IHC	62/146 (43%)	<48 months	NS/NS
Hanzal [55]	IHC	29/117 (25%)	91 months	NS/NA
Thorlacius [146]	CDGE	18/109 (17%)	32 months	NA/NA
Andersen [3]	CDGE/ IHC	35/163 (26%)	38 months	NN: NS/NS NP: 0.05/NS
Lipponen [90]	IHC	113/193 (58%)	13.8 years	NS/NS
Visscher [151]	IHC	NN: 6/34 (18%) NP: 18/43 (42%)	52 months	NN: NS/NA NP: 0.03/NA
Barnes [5]	IHC	NN: 20/103 (19%) NP: 18/92 (20%)	10 years 10 years	NA/0.09 NA/0.001
Hurlimann [65]	IHC	NN: 31/91 (34%) NP: 38/99 (38%)	81 months	NS/NS NS/NS
Marks [99]	IHC	NN: 39/147 (27%) NP: 17/83 (20%)	5 years	0.03/0.01 NS/NS
Caleffi [23]	DGGE	NN: 12/78 (15%) NP: 30/111 (27%)	48 months	NA/NS
Domagala [33]	IHC	NN: 44/127 (35%) NP: 24/100 (24%)	87 months	NA/NS
Haerslev [53]	IHC	148/490 (32%)	10 years	NS/NS

tastases (node-negative), whereas 16 studies reported on mixed series of node-negative and node-positive patients. Whenever possible, we have listed the results of the latter studies stratifying patients by lymph node status, because of its great importance in guiding patient management. The patient follow-up ranged from a minimum of 28 months to more than 10 years; only five studies however, had a follow-up of at least 10 years. Disease-free (DFS) and overall survival (OS) were reported by most, but not all authors; multivariate, analysis was available in about half of these investigations [2, 3, 5, 33, 36, 51, 53, 67, 90, 99, 136, 145, 146]. Most importantly, data on p53 genotype were available, at least in part, in only 5 of the 22 papers reviewed: the remaining investigations relied exclusively on immunohistochemical data. Furthermore, only three series [11, 123, 137] comprised patients who did not receive any postoperative adjuvant therapy, whereas in the other studies this information was not provided or a variable percentage of patients was treated postoperatively with either chemotherapy or radiation therapy or both (see below for a more detailed discussion of this point).

As a consequence of the above limitations a definite conclusion on the prognostic value of p53 aberrations in breast carcinoma is still not possible. Some additional comments may be useful in analysing the data presented in Table 1. Among the studies where molecular techniques were used to detect p53 gene mutations, Andersen et al. [3] found an excellent concordance between the prevalence of mutations and of p53 protein accumulation (21% and 22% respectively), whereas Allred et al. [2] reported a marked difference in the subgroup of patients for whom both p53 gene mutation and p53 protein accumulation data were available (14% and 47%, respectively). Overall, in the five papers in which p53 gene status has been assessed, the prevalence of mutations ranged between 14% and 22%. However, several authors using IHC have indicated a prevalence of p53 accumulation ranging from 14% up to 58%. Thus a significant fraction of tumours is expected to display p53 protein accumulation without any corresponding p53 mutation. It is noteworthy that the poor correlation between gene mutation and protein accumulation that has been generally reported, and reemphasized in the techniques section [52], is true of breast cancer and, as shown below, of lung and colorectal cancer.

Several studies have shown that p53 accumulation may be used for prognostic purposes, being significantly correlated with a worse survival, although in one series p53 accumulation was associated with a favourable re-

Table 2 Lung cancer (*FCM* flow cytometry; other abbreviations as in Table 1)

First author [ref. no.]	Method	Number positive (%)	FU (mean)	OS
Quinlan [120]	IHC	49/114 (43%)	>3 years	0.001
McLaren [102]	IHC	70/125 (54%)	31 months	NS
Morkve [106]	FCM	86/112 (77%)	>4 years	NS
Horio [64]	SSCP	35/71 (49%)	NA	0.014
Mitsudomi [103]	SSCP	51/120 (43%)	16 months	0.01
Ebina [34]	IHC	28/123 (23%)	>4 years	0.0027

lapse-free survival [90]. It is, however, important to note that multivariate analyses have been used to confirm the independent prognostic role of p53 accumulation in only nine investigations [2, 3, 5, 36, 51, 99, 137, 145, 146]; the majority of these studies included series of patients with a relatively short follow-up. The discrepancy between gene mutation and protein accumulation may underscore the fact that p53 protein may be stabilized by mechanisms other than mutation. These alternative mechanisms causing p53 inactivation may be actually more critical in determining tumour behaviour than gene mutation. The only way to assess the clinical impact of different types of p53 aberrations will be to study p53 systematically both at the gene and protein level, in the same tumours, within the framework of large clinical trials. This will allow identification of subgroups of patients, if any, in which the assessment of p53 status will help to improve the results of adjuvant therapy.

Lung cancer

The studies on lung cancer are summarized in Table 2. Although a smaller number of patients have been investigated by a few groups, the results are much more homogeneous than in breast cancer. Except for one series [102] in which a few small-cell carcinomas were included, the large majority of tumours investigated were non-small-cell lung carcinomas. The length of follow-up, although not particularly long, still may be considered adequate, due to the biological and clinical aggressiveness of these tumours. Furthermore, two of the six investigations have used SSCP analysis to determine the tumour p53 genotype. Unfortunately, few details were given as to whether or not patients received any adjuvant therapy.

With a single exception [102], p53 aberrations proved to be an unfavourable prognostic indicator in univariate analysis. Four studies confirmed this result with multivariate analysis [34, 64, 103, 120]. In three series the prognostic significance was established in stage I and II patients [34, 64, 120], whereas in one series [103] p53 mutations were associated with a worse survival in advanced (stage III and IV) but not in early stages of disease (I and II). Morkve et al. [106] reported the surprising and unexplained finding that tumours with low and moderate expression of p53 protein were more aggressive than the negative tumours and those with high p53 protein expression.

Overall, the available data suggest that p53 aberrations are significantly associated with a worse survival in non-small-cell lung cancer. Given the occurrence of discrepant results, after accounting for the disease stage, additional studies are still needed. Furthermore, the significance of different types of p53 aberrations will have to be specifically investigated. Although in lung cancer, at variance with tumours arising in other organs, there are fewer investigations comparing p53 gene mutations and p53 protein accumulation, discrepancies to exist. Marchetti et al. [97] reported only a 54% concordance between IHC and SSCP data in a series of non-small-cell lung carcinomas. Furthermore, nonsense and splicing mutations, leading to a "p53 null" phenotype, may be particularly frequent in lung carcinoma [10].

Gastrointestinal cancer

Investigations pertaining to p53 aberrations in tumours of the gastrointestinal tract are summarized in Table 3. Colorectal carcinomas have been most extensively studied, followed by oesophageal and gastric carcinomas.

Of the four studies on oesophageal cancer using IHC techniques to demonstrate p53 accumulation, three showed an unfavourable prognostic significance in univariate survival analysis. Multivariate analysis, however, was not used to confirm these results. All three investigated Japanese and Chinese patients. The same conclusion was not supported by a European study, in which p53 accumulation was not related to poor survival. Whether these discrepancies depend on genetic and aetiological factors which affect differently Orientals versus Americans and Western Europeans remains to be assessed. Our own preliminary findings on 71 Italian patients with oesophageal carcinoma in whom data were obtained on p53 genotype (SSCP analysis) and on p53 accumulation also failed to demonstrate an association between p53 aberrations and poor survival (Coggi G, Roncalli M, Bosari S, Graziani D, Borsani G, Bossi P, Buffa R, Ferrero S, Peracchia A, Segalin A, Bonavina L, Piazza M, Viale G (1995) p53 gene product accumulation and mutations in oesophageal cancer: correlations with tumour type, grade, stage and survival analysis. Proceedings of the XV European Congress of Pathology, Copenhagen, Denmark).

Two studies reported that p53 accumulation is associated with worse survival in gastric cancer, demonstrated by univariate and multivariate analysis [72, 100]. In one investigation [139] a shorter combined OS and DFS was observed. Two studies failed to confirm this conclusion,

Table 3 Gastrointestinal cancer (*r* range)

First author [ref. no.]	Method	Number positive (%)	FU (mean)	OS
Oesophagus				
Furihata [49]	IHC	24/71 (34%)	5 years	0.05
Shimaya [134]	IHC	56/105 (53%)	1.7 years	0.05
Sarbia [126]	IHC	137/204 (67%)	40 months	NS
Wang [153]	IHC	65/100 (65%)	>10 years	0.005
Stomach				
Martin [100]	IHC	72/125 (58%)	1–113 months (r)	0.02
Starzynska [139]	IHC	26/55 (47%)	2 years	0.001
Kakeji [73]	IHC	52/96 (54%)	1–2 years	NS
Hurlimann [66]	IHC	28/72 (39%)	3 years	NS
Joypaul [72]	IHC	94/206 (46%)	>5 years	0.01
Colorectum				
Scott et al. [130]	IHC	22/52 (42%)	35 months	NS
Remvikos [122]	FCM	52/78 (67%)	42 months	0.03
Sun [140]	IHC	73/293 (25%)	>5 years	<0.001
Starzynska [139]	IHC	49/107 (46%)	12 months	0.001
Yamaguchi [15]	IHC	61/100 (61%)	6–48 months (r)	0.05
Bell [7]	IHC	45/100 (45%)	34 months	NS
Bosari [14]	IHC	99/197 (50%)	>5 years	0.0017
Hamelin [54]	DGGE	44/85% (52%)	47 months	0.003
Nathanson [111]	IHC	52/84 (62%)	>5 years	NS
Tanaka [141]	IHC	23/36 (64%)	>2 years	NA
Zeng [158]	IHC	50/107 (47%)	62 months	0.02 (DFS)
Bosari [17]	SSCP	74/126 (59%)	>5 years	NS

although limited follow-up was available. Interestingly, some data [66] suggest that p53 accumulation may be involved only in the intestinal type of gastric cancer, but not in the diffuse type. This has not been confirmed by Joypaul et al. [72]: in their series p53 accumulation was not correlated with specific histological types.

At least 12 investigations have evaluated p53 aberrations and prognosis in colorectal cancer. All but two used IHC techniques. Follow-up length was variable, often less than 5 years. The results of these investigations are disappointingly discordant. Some studies used selected patient populations: for instance Zeng et al. [158] investigated only patients with stage III colorectal carcinoma and normal preoperative levels of carcinoembryonic antigen (CEA). In this highly selected group of patients, p53 accumulation turned out to be an independent prognostic indicator. The two investigations comprising the largest number of patients with long-term follow-up (>5 years) reported a significantly worse prognosis for patients with tumours displaying cytoplasmic, but not nuclear, p53 accumulation [14, 140]. Molecular analysis of the tumours in our own series [17], however, revealed that cytoplasmic p53 accumulation is not related to p53 gene mutations, being instead significantly associated with wild-type p53 gene. This re-emphasizes the possible role of mechanisms other than gene abnormalities in inducing loss of p53 functions or the oncogenic effects of a true overexpression of the gene with a consequent "gain of function". The two investigations that evaluated the p53 genotype with molecular techniques have also provided conflicting results.

It should be noted that tumorigenesis in the large bowel has been extensively investigated, and the importance of multiple genetic defects has been demonstrated (for review see [9, 39]). The genes involved in large bowel carcinogenesis and tumour progression include APC, hMSH2, hMLH1, hPMS1, hPMS2, k-*ras*, DCC and p53 [9]. Furthermore, genes regulating programmed cell death (apoptosis), such as bcl-2, may also play a role in colorectal tumorigenesis [15, 20]. Among this cascade of genetic alterations, p53 mutations most likely play a crucial role at the transition from adenoma to carcinoma. It would be important to investigate the combined role of several of these genetic defects, in order to unveil how they affect tumour behaviour. Data on multiple oncogenes and tumour suppressor genes in the same clinical series are still lacking. Mutations of k-*ras* oncogene have been reported to affect survival in colorectal cancer patients [8, 41]. Bell et al. [7] evaluated both k-*ras* mutations and p53 accumulation and found that only a combination of the two is significantly correlated with tumour aggressiveness and patient survival. The fact that the sum of diverse genetic alterations affects tumour progression and the clinical outcome is indirectly supported by the demonstration that fractional allele loss is an unfavourable prognostic parameter in colorectal cancer [75]. Clearly more studies are needed to address this tissue specifically.

Genitourinary cancer

Overall, tumours of the genital and urinary tracts have received less attention than those of the breast and the gastrointestinal tract. In particular, with a single exception [149], all investigations have been performed using exclusively IHC methods thus far.

Table 4 Genitourinary cancer (*IFA* immunofluorometric assay, *PFI* progression-free interval)

First authors [ref. no.]	Method	Number positive (%)	FU (mean)	OS	Notes
Kidney					
Uhlman [147]	IHC	49/172 (28%)	61 months	0.003	For non-metastatic tumours
Lipponen [91]	IHC	41/124 (33%)	9.4 years	NS	
Bot [18]	IHC	32/100 (32%)	39 months	NS	
Bladder					
Sarkis [127]	IHC	25/43 (58%)	119 months	<0.003	(PFI), T1
Furihata [48]	IHC	32/90 (35.6%)	1–12 years (r)	<0.05	
Lipponen [89]	IHC	62/212 (29%)	2.5–5 years (r)	0.015	
Vet [149]	SSCP	8/47 (17%)	>36 months	0.001	
Sarkis [128]	IHC	15/55 (45%)	124 months	0.008	Tis
Esrig [38]	IHC	101/243 (41.5%)	6 years	0.001	
Endometrium					
Ito [68]	IHC	47/221 (21%)	41 months	0.0001	
Lukes [96]	IHC	21/100 (21%)	3.4 years	0.001	(DFS)
Nielsen [112]	IHC	36/109 (33%)	46 months	0.01	(DFS+OS)
Khalifa [79]	IHC	6/69 (9%)	NA	NS	
Reinartz [121]	IHC	37/128 (29%)	NA	<0.0017	
Ovary					
Marks [98]	IHC	54/107 (50%)	NA	NS	
Bosari [13]	IHC	44/98 (45%)	42 months	0.0025	
Hartmann [57]	IHC	177/284 (62%)	7 years	0.05	
van der Zee [157]	IHC	31/89 (35%)	>6 years	0.0001–0.003	(DFS+OS)
Levesque [85]	IFA	39/90 (43%)	22 months	0.06	for grade I and II
Prostate					
Visakorpi [150]	IHC	8/137 (6%)	>10 years	<0.001	
Fox [43]	IHC	6/45 (13%)	>36 months	NS (0.079)	Stage A1
Vesalainen [148]	IHC	21/139 (15%)	12 years	NS	T1–2
Shurbaji [136]	IHC	23/109 (21%)	3.84 years	<0.03	(DFS), Gleason 2–7

From the review of the published series, it appears that p53 accumulation is consistently associated with a reduced progression-free interval (in Tis and T1 stages) and worse survival in bladder carcinomas. Its independent prognostic value has been confirmed in five of seven investigations by multivariate analysis. Interestingly, however, the single study exploring p53 gene status by SSCP analysis failed to document an independent prognostic value for gene mutations [149].

Renal adenocarcinomas show p53 immunoreactivity in approximately one-third of the cases, but this is not correlated with survival, with the possible exception of non-metastasizing tumours [147]. In these cases, p53 accumulation has been reported (in multivariate analysis) to be a more powerful prognostic parameter than tumour size and grade.

In endometrial adenocarcinomas, p53 immunoreactivity has been shown to correlate significantly with a worse DFS and OS, but its independent prognostic value has been confirmed by multivariate analysis in only one study [68]. Interstingly, Khalifa et al. [79], at variance with all other investigations, failed to document any p53 immunoreactivity in endometrioid-type adenocarcinomas, while immunostaining was restricted to 6 of 24 non-endometrioid carcinomas.

Several studies have suggested that p53 accumulation is related to a worse prognosis in at least certain subsets of ovarian carcinoma patients. However, lack of independent prognostic significance by multivariate analysis has been reported consistently, most likely because in these tumours p53 immunoreactivity is strictly correlated with the histological grade. However, when patients have been stratified on the basis of the tumour grade, p53 accumulation has retained its adverse prognostic effect in better-differentiated neoplasms [85].

Finally, the correlation between p53 immunoreactivity and survival in prostatic carcinoma patients is controversial. Given the uncertainty about the most appropriate treatment for early-stage carcinomas, it would be of paramount importance to identify reliable predictors of progression in these patients. The single study [136] which documented an independent prognostic role for p53 included only 26 stage-A carcinomas, 5 of which were immunoreactive. In multivariate analysis, p53 accumulation correlated with a shorter DFS only for well and moderately differentiated (Gleason score 2–7) tumours.

Other tumour types

Squamous cell carcinomas of the head and neck region have been extensively investigated for p53 aberrations, using both immunocytochemical and molecular analyses. The prevalence of abnormal p53 accumulation ranges

between 37% [44] and 78% [50] of the cases, while gene alterations have been detected in 43% [19] and 69% of primary and metastatic tumours. Interestingly, both protein accumulation and gene alterations have been detected consistently in premalignant and non-invasive lesions [19, 74, 135], thus emphasizing the role of p53 aberrations in the early steps of head and neck tumorigenesis.

p53 abnormalities did not show any correlation with clinicopathological variables, including stage and grade of the tumours [40, 50], nor have they proved to be prognostically useful in uni- or multivariate analyses of survival [1, 44].

Investigations on central nervous system tumours have repeatedly shown that p53 mutations and/or accumulation are confined to neoplasms showing astrocytic features (astrocytomas, anaplastic astrocytomas and glioblastomas) and to oligodendrogliomas [21, 81, 105, 124]. p53 accumulation has been reported to correlate with tumour grade by some authors [21, 70], but not by others [81, 105]. Also, the prognostic significance of p53 accumulation has been emphasized in astrocytic neoplasms by Jaros et al. [70], but denied by a subsequent investigation [105]. In oligodendrogliomas, p53 accumulation in more than 75% neoplastic cells has been shown to significantly correlate with shorter survival in univariate analysis [81].

p53 aberrations and adjuvant therapy

Wild-type p53 can direct cells into the programmed cell death pathway. This has been shown for both haemopoietic and epithelial cell lines [131, 156]. Furthermore, while normal thymocytes with wild-type p53 undergo apoptosis after exposure to DNA-damaging agents such as radiation or etoposide, thymocytes lacking p53 (from knock-out mice) are protected and do not undergo apoptosis under the same conditions [27, 94]. An increasing body of evidence suggests that anticancer agents act by inducing apoptosis [31, 77] and that p53 is a crucial component of the pathway leading to apoptosis in transformed cells exposed to anticancer agents [93, 142]. The role of p53 in inducing tumour cell apoptosis has been confirmed by Lowe et al. [95] using a fibrosarcoma cell line with or without wild-type p53 ($p53^{+/+}$ and $p53^{-/-}$, respectively), transplanted in nude mice. Tumours derived from $p53^{+/+}$ cells regressed after gamma radiation and Adriamycin exposure and contained a high proportion of apoptotic cells, whereas $p53^{-/-}$ tumours were resistant to the same treatment, continued to grow and showed few apoptotic cells. Furthermore, the adenovirus-mediated transfection of wild-type p53 in human cancer cells transplanted in nude mice induces chemosensitivity, as demonstrated by massive tumour cell apoptosis after cisplatinum administration [46]. Another possible mechanism that can influence susceptibility to chemotherapy of tumours harbouring mutated p53 is the induction of the multidrug resistance gene (MDR), which confers resistance to a variety of anticancer agents [26].

Despite these compelling experimental data, the evidence that p53 gene status, or p53 protein accumulation, actually affects the clinical response to chemotherapy and radiotherapy of human tumours is scanty. In some of the papers discussed in the preceding sections only suggestions, and not hard data, are put forward. Indeed, as underlined in the breast cancer section, the admixture of patients being treated with either chemotherapy or radiotherapy has not facilitated our understanding of this important issue. Overall, it appears that in breast cancer the unfavourable prognostic role of p53 aberrations may be stronger in node-positive than in node-negative patients. Since the latter have been subjected to a variety of treatment regimens and many had only surgical therapy, whereas the former have almost always received adjuvant therapy, the results support, but do not definitely prove, the suggestion that p53 aberrations may influence the response to post-surgical therapy. Recently Tetu et al. [144], investigating a series of 847 node-positive breast cancer patients, showed that p53 accumulation predicted a worse survival in patients treated with adjuvant chemotherapy or hormone therapy, but not in those patients who did not receive such therapies. Conversely, Muss et al. [109] have shown that breast cancer patients with tumours expressing high levels of the c-*erb*B-2 (neu) oncogene respond favourably to adjuvant therapy, whereas p53 accumulation was not related to the response to therapy in the same series of patients. In other organs, p53 aberrations have been linked with poor survival in patients presenting with advanced disease stages and treated with anticancer chemotherapy. Again, these results suggest that p53 status may be important for the response to adjuvant therapy, but conclusive evidence is lacking.

Conclusions and future perspectives

There is no doubt that the p53 gene plays a critical role in suppressing tumorigenesis, probably by minimizing the mutagenic effects of DNA damage [87]. Such a physiological role is disrupted in a large number of human tumours: although mutations are responsible in the majority of cases [52], other mechanisms inducing p53 loss of function have been described [87], and may occur quite frequently [17, 28].

Are tumours harbouring p53 aberrations more aggressive than those that have been transformed via different mechanisms? Notwithstanding the large number of studies on the subject, we should admit that a definite answer to this question is not yet available. As discussed in the preceding sections, there are no tumour types in which there is complete agreement on the independent unfavourable prognostic significance of p53 aberrations. Although recently, in reviews and editorials [24, 47], it has been proposed that p53 aberrations are indeed an unfavourable prognostic indicator, it is noteworthy that the literature cited has several omissions, with particular reference to those investigations which do not support this

conclusion. Several discrepancies may be explained by the different techniques used in the studies reviewed: the predominance of IHC-based studies may have created more than a bias. Certainly, additional data on cancer patients in whom mutations have actually been demonstrated by molecular techniques are needed.

The clinical significance of the combined effect of abnormalities of different oncogenes and tumour suppressor genes has also to be assessed. The role of the deregulated expression of oncoproteins in different steps of the metastatic cascade, is uncertain. For instance, in experimental systems, wild-type p53 stimulates thrombospondin production [29], whereas mutated p53 may increase vascular endothelial growth factor synthesis [80], thereby possibly modulating tumour angiogenesis. It is not known whether this angiogenic modulation plays a significant role in human tumour progression.

p53 has come to the forefront of research and clinical oncology. Undoubtedly many advances have been made, but many more studies are still needed to shed light on one of the most fascinating and promising medical issues of our time. However, to provide consistent and reproducible results, useful for patient care and for the advancement of medical knowledge, standardized study designs and protocols should be implemented. The lesson we have learnt from our critical review of the literature on the subject can be summarized in the following recommendations:

1. Large and homogeneous series of patients in whom long-term follow-up is, or will be, available should be investigated. A large number of cases is necessary to allow stratification of patients according to pertinent clinicopathological data and to study the effects of p53 abnormalities both in the entire cohort of patients and in the diverse strata. Homogeneity of the treatment regimens (with particular reference to postoperative therapy) should be a feature of the series under study. The results from several studies have shown that p53 aberrations tend to be more prevalent in tumours presenting in advanced pathological stages, and in poorly differentiated tumours with high proliferative rates. The conclusions of future studies should therefore be supported by multivariate statistical analysis in order to assess the independent prognostic value of p53 abnormalities in these series of patients.
2. Both p53 gene and protein status should be analysed. This can also be accomplished in retrospective series of routinely fixed and embedded material, using well-established methods. Molecular techniques that have been extensively tested (such as SSCP or DGGE) should be preferred. Sequence analysis should also be performed in order to confirm the occurrence of p53 gene abnormalities and to evaluate the possible clinical significance of different genetic changes. Indeed not all p53 mutants are equivalent and distinct protein domains have different functions [62, 125]. It is therefore conceivable that different genetic changes may induce diverse biological and clinical effects. Such investigations should encompass all p53 exons, and not be restricted to exons 5–8. Though most missense mutations are actually clustered in these latter exons, other gene abnormalities (microdeletions and microinsertions, nonsense mutations) are relatively common in different exons of the gene. Hartmann et al. [58] recently reported a 9% increase (from 33% to 42%) in the number of breast carcinomas carrying p53 gene abnormalities, when the analysis was extended to exons 2–4 and 9–11. All the additional 18 mutations outside of exons 5–8 were null mutations, resulting in truncated or garbled proteins, which are undetectable by immunocytochemistry.

The immunocytochemical localization of p53 protein must rely on standardized techniques, using well-characterized antibodies. The optimal dilution of these reagents must be defined by accurate checkerboard titrations. Nuclear and cytoplasmic immunoreactivity should be separately recorded and correlated with the clinicopathological features and the clinical outcome. The immunocytochemical results should be scored quantitatively, taking into account the percentage of immunostained tumour cells. It must be remembered that molecular techniques will document the occurrence of gene abnormalities only if the cells carrying the abnormal gene represent at least 5–10% of the neoplastic cell population. This should be considered when the immunocytochemical findings are correlated with molecular data.
3. The role of p53 as a biomarker of susceptibility to different therapeutic regimens should be assessed. To accomplish this goal, ad hoc clinical trials are not necessary, however, and the assessment of p53 abnormalities can be added to the design of clinical trials aimed at evaluating specific therapeutic approaches. This should greatly facilitate the feasibility of the study and reduce funding requirements, whilst still providing critical information on the response to anticancer therapy. A few such large studies, involving multiple institutions, and with the use of a centralized testing facility, could provide definite answers in a timely fashion.

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