Histol Histopathol (1999) 14: 279-284

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Invited Review

Clinical applications of detecting dysfunctional p53 tumor suppressor protein

I.O. Baas[1], R.H. Hruban[2] and G.J.A. Offerhaus[1]

[1]Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands and

[2]Departments of Pathology and Oncology, Johns Hopkins Medical Institutions, Baltimore, MD, USA

Summary. The *p53* gene encodes for a protein, p53, which plays a critical role in controlling the cell cycle, in DNA repair and in programed cell death (apoptosis). *p53* is one of the most frequently mutated genes in human neoplasms and a variety of techniques have been developed to detect these mutations. These range from advanced molecular-genetic analyses to immunohistochemical staining for the p53 protein. This review will summarize our current understanding of the function of p53 as well as current methods to detect dysfunctional p53 and the clinical value of such analyses.

Key words: *p53*, Tumor suppressor gene, Cell cycle, Neoplasia, Cancer

Introduction

The *p53* tumor-suppressor gene is the most frequently altered gene in human malignancies (Levine, 1992; Wynford-Thomas, 1996). It is located on the short arm of chromosome 17 and encodes a 53 kD nuclear phosphoprotein called p53. Knowledge of the biological function of p53 and the molecular mechanisms through which p53 exerts its function will not only provide a better understanding of the process of carcinogenesis, but will also help identify new molecular targets which can be used to aid in the diagnosis of cancer and which may be of value in the management of cancer patients. Since *p53* is functionally inactivated in the majority of human cancers, this may have major clinical implications. The authors will review the basic features of p53 function and its clinical relevance.

Functions of wild-type *p53*

The prominent role of p53 dysfunction in tumorigenesis can be understood if one appreciates the importance of wild-type p53 in cell cycle regulation (Fig. 1). The main function of wild-type p53 is to protect the cell against DNA damage by preventing replication following DNA damage. Cell division with damaged DNA would lead to fixation of the damage, resulting in increased genomic instability and sensitivity of the cell to further DNA damage. Because of this protective function, p53 has been nicknamed the "*Guardian of the genome*" (Lane, 1992).

Wild-type p53 operates through at least three different molecular mechanisms (Harris, 1996) (Fig. 1). First, p53 acts as a checkpoint for DNA damage in cell cycle progression at the transition from G1 to S phase (Nishida et al., 1997). If the DNA is damaged, p53 accumulates and acts as a transcription factor for *p21* (also called *Cip1* or *waf1*). p21 is a nuclear protein that can bind to cyclin-dependent kinases and thereby inactivate them (Xiong et al., 1993). Cyclin-dependent kinases are enzymes involved in the control of cell cycle progression. Interaction of p21 with the cyclin-dependent kinases, guided by p53, results in cell cycle arrest. This arrest creates time for DNA repair to occur prior to the cell entering S phase and thus damaged DNA will be repaired before it is replicated (Fig. 1) (Kern, 1994). Second, in addition to providing time for DNA repair, p53 is also directly involved in DNA repair itself. p53 turns on the DNA repair machinery in the cell by interacting with several cellular proteins (Harris, 1996). These proteins include: "Proliferating Cell Nuclear Antigen" (PCNA, required for copying and repair of DNA), "Excision Repair Cross-Complementing 3 protein" (ERCC3, an excision repair enzyme that participates in the removal of damaged DNA) and "Growth Arrest DNA Damage inducible protein" (GADD45, which complexes with PCNA and thereby stimulates its activity). Finally, if DNA damage is severe, p53 pushes the cell into programmed cell death (apoptosis). By promoting apoptosis, p53 ensures that severely damaged DNA will not be duplicated and that the damage will not be fixed into genomic material. For this function, p53 interacts with two of the main mediators of apoptosis, Bcl-2 and Bax (Harris, 1996).

Offprint requests to: Dr. Ralph H. Hruban, MD., Departments of Pathology and Oncology, Johns Hopkins Medical Institutions, 600 N. Wolfe Street, Baltimore, MD 21287, USA. Fax: 1-410-955-0115. e-mail: rhruban@welchlink.welch.jhu.edu

The exact molecular pathway that leads from DNA damage to p53 accumulation is unknown (Harris, 1996). *In vitro* studies have shown that p53 protein has sequence-specific DNA binding capacity (Kern et al., 1991). The DNA binding domain of the p53 protein is encoded for by exons 5-8 of the p53 gene and it consists of regions which have been highly conserved through evolution. p53 needs this sequence-specific DNA binding in order to exert its function. Mutation in one of these exons results in conformational changes at the binding sites, reducing the sequence specific binding capacity of the p53 protein.

The identification of specific DNA binding sites in the p53 protein, and the obvious symmetry found in these binding sites, suggested that p53 acts as a tetrameric protein (Vogelstein and Kinzler, 1992). Biophysical studies support this hypothesis. A tetrameric configuration of the p53 protein would also explain the "dominant-negative" effect of mutant p53: the mutant and wild-type proteins form a tetramer. Tetrameric complexes of wild-type and mutant p53 protein cannot bind with DNA and therefore the complex is non-functional. Although p53 is a tumor suppressor gene, it may also exert a dominant effect, similar to oncogenes. This dominant-negative effect is not shared by all p53 mutations and some mutations exert a more potent dominant-negative effect than do others (Vogelstein and Kinzler, 1992).

Recently, the first human *p53* homologue, called *p73*, was discovered (Dickman, 1997; Oren, 1997). *p73* is located on a region of chromosome 1, which is often deleted in neuroblastomas, suggesting that *p73* may be a tumor-suppressor gene. Furthermore, p73 is predicted to have a significant amino acid sequence similar to p53, especially in the amino acid residues implicated in the sequence specific DNA binding of p53. p73 has also been shown to activate the transcription of p53-responsive genes, amongst others p21, and inhibit cell growth in a p53-like manner by inducing apoptosis (Jost et al., 1997). The discovery of a *p53*-homologue raises the possibility of the existence of additional *p53* family members and also suggests that p53 function could be restored by recruiting p73.

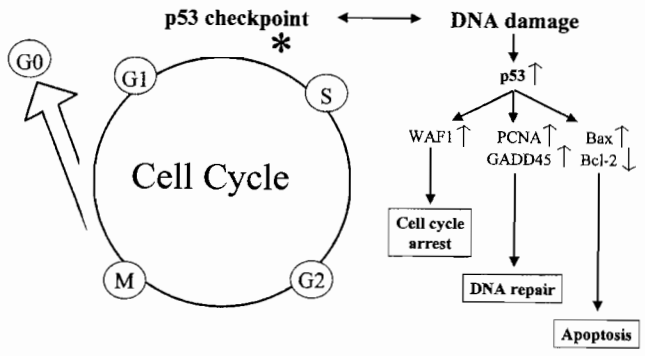

Fig. 1. p53 in cell cycle regulation

In summary, p53 plays a key role in several central cellular processes through the transcriptional activation of genes and by interacting with proteins involved in cell cycling, DNA repair and apoptosis. In this way, p53 acts as the "*Guardian of the genome*," protecting against DNA damage. Loss of the protective function of p53 leads to an inadequate response to DNA damaging agents, resulting in genomic instability of the cell.

Loss of wild-type p53

Although *p53* is the most frequently altered gene in human malignancies, loss of p53 function appears to be a late event in tumorigenesis in many tumor types (Baker et al., 1990). This raises the question of how can a key player in carcinogenesis only be involved in the final stages of this process? The answer seems to be that p53 functions as an emergency brake, used only in stressful situations as a protective measure (Kinzler and Vogelstein, 1996). In the every day life of normal cells, p53 only plays a minor role. This is illustrated by the fact that *p53* knock-out mice are initially viable and healthy. Only later in life do they show an increased susceptibility for tumor development (Donehower et al., 1992). In the event of cellular stress, p53 becomes important as a regulator of the cell cycle. Wild-type p53 is activated in response to cellular stresses, such as DNA damage or an increased proliferation rate in early-stage neoplasia. Here, functional p53 limits cell growth and tumor progression by inhibiting cell cycle progression or by inducing apoptosis. In contrast, dysfunctional p53 would allow further tumor expansion; stress offers a selective advantage for cells containing *p53* mutations (Vogelstein and Kinzler, 1992). Thus, a condition of cellular stress must exist before p53 becomes important to tumor progression. This may explain why *p53* mutations are generally implicated late in tumorigenesis.

In normal cells, two alleles of the *p53* gene are present, a paternal and a maternal copy. The presence of one normal allele is sufficient for p53 to exert its protective function. Thus, at the molecular-genetic level, two distinct events are required to inactivate both *p53* alleles, and p53 function (Ireland et al., 1997). Usually, one *p53* allele is inactivated by an intragenic mutation and the other allele is completely lost. Eighty percent of the intragenic mutations in *p53* are missense mutations, causing one amino acid to be substituted for another (Harris and Hollstein, 1993). These usually result in conformational changes in the p53 protein. In addition to point mutations, allelic loss, rearrangements and small deletions of the *p53* gene have been detected in human neoplasms (Hollstein et al., 1991).

Mutation in the *p53* gene can also be inherited. The Li Fraumeni Syndrome (or Familial Cancer Syndrome), first described in 1969, is characterized by the autosomal dominant inheritance of a predisposition to multiple primary neoplasms in children and young adults, notably breast carcinoma, osteosarcoma, brain tumors and soft tissue sarcomas. Affected family members carry a

Table 1. Proteins interacting with p53 protein.

CELLULAR PROTEINS	VIRAL PROTEINS
MDM-2	Simian Virus 40 - Virus Large T antigen
ERCC3	Epstein-Barr Virus - EBNA 5
70 kD Heat Shock Protein	Hepatitis B Virus - X
	Human Cytomegalovirus - IE84
	Human Papilloma Virus 16 and 18 - E6
	Adenovirus 5 and 12 - E1b

germline mutation of the *p53* tumor-suppressor gene (Malkin et al., 1990; Srivastava et al., 1990). These mutations are usually in the highly conserved regions of exons 5-8 of *p53*, similar to the somatic mutations seen in sporadic tumors (Kleihues et al., 1997). Since carriers of the mutation inherit only one functional *p53* allele, following the Knudson model (Knudson, 1971), only one molecular-genetic event is needed to abrogate the protective function of p53 in these carriers.

In addition to molecular-genetic events, p53 function can also be lost is through protein-protein interactions. A variety of proteins have been identified that can bind to the p53 protein and thereby impair its function (Hall et al., 1996) (Table 1). The human homologue of the *Mouse Double Minute-2* (MDM-2) gene, located on chromosome 12q, is known to disrupt the p53 tumor suppressor pathway in this manner (Garcia et al., 1997). This cellular oncogene can be transcriptionally activated by wild-type p53 protein. MDM-2 protein subsequently complexes with p53 protein, thereby inactivating it and preventing further transcription. In this way, the *MDM-2* gene is autoregulated under physiologic conditions (Wu et al., 1993). Amplification of the *MDM-2* gene is found in approximately one third of human sarcomas (Oliner et al., 1992; Hung and Anderson, 1997). Overexpression of the *MDM-2* oncogene product, caused by amplification of the gene, interferes with the balance of this auto-regulation and results in uncontrolled cell growth and malignant transformation. Of special interest is the fact that viral proteins can also bind to p53 and impair p53 protein function (Table 1). These protein interactions can either stabilize wild-type p53 protein or accelerate its degradation, resulting in loss of p53 function and uncontrolled cell growth. The ability of certain viruses to knock out p53 function offers a molecular substrate for the known oncogenic potential of some viruses in humans, e.g. Human Papilloma virus in cervical carcinoma and Epstein-Barr virus in Burkitt lymphoma and nasopharyngeal carcinoma. Recently, the interaction of Cytomegalovirus gene products with p53 has been implicated in the smooth muscle cell proliferation in seen coronary restenosis following balloon angioplasty (Speir et al., 1994).

In summary, tumor development can be instigated by a number of factors, resulting in cellular stress. This creates a selective advantage for cells with dysfunctional p53, since p53 plays an important role in controlling growth in stressed cells. p53 dysfunction is therefore generally not implicated in tumor initiation, but instead in the expansion of an already existing neoplasm. The *p53* gene can be abrogated at the molecular-genetic level by two distinct events resulting in inactivation of both *p53* alleles. Functional inactivation of p53 can also occur by binding of p53 protein to other proteins, either endogenous or viral.

Clinical significance

This understanding of the funciton of p53 can be used to help in patient management in a number of ways. First, the p53-status of a cell could aid in cytologic and histologic diagnoses. The absence of clonal alterations in the *p53* gene could help distinguish between reactive and neoplastic cells (Hall et al., 1991). Second, once the diagnosis of a neoplasm is established, p53-status may serve as a prognostic marker (Dowell and Hall, 1995). A number of studies have assessed the potential value of p53 dysfunction as an indicator of poor prognosis. Most of these were studies in which a cohort of patients with a particular neoplasm was analyzed for p53 function and then the survival of patients with neoplams with dysfunctional p53 was compared to the survival of patients with neoplasms with functional p53. It is, however, important to realize that the methods used in these studies vary substantially with respect to total number of patients and in the techniques used to assess p53 function. In general, the more patients included in a study, the more likely is the study to show that p53 dysfunction is a prognostic factor. This indicates that, for most tumors, p53 dysfunction is probably only a relatively weak prognostic factor (Dowell and Hall, 1995). The one exception to this may be superficial transitional-cell bladder carcinoma. In these neoplasms the prognostic value of p53 protein expression has been demonstrated consistently (Soini et al., 1993; Esrig et al., 1994; Serth et al., 1995).

Third, the p53-status of a neoplasm may help to determine which kind of anticancer therapy will be most successful. Treatment with radiation and many chemo-therapeutic agents ultimately results in apoptotic death of the neoplastic cells. *In vitro* and *in vivo* studies have shown that wild-type *p53* is required for this induced apoptotic cell death (Clarke et al., 1993; Lowe et al., 1993, 1994; Kinzler and Vogelstein, 1994). It is therefore not surprising that radiation and chemotherapy are known to be highly effective in primary neoplasms that only rarely exhibit *p53* mutations (testicular cancer, neuroblastoma, Wilm's tumor and childhood lympho-blastic leukemia), while, several of the primary tumors that frequently harbor p53 mutations (malignant melanoma, cancer of the lung, esophagus, stomach, colon, bladder and prostate) are generally insensitive to such treatments. These data suggest that p53 is important in the response of neoplastic cells to genotoxic therapy. p53-status could function as a discriminator to identify those neoplasms likely to respond favorably to genotoxic therapy. Finally, the rapid discovery of the various

components of the p53 tumor suppression pathway offers potential new prospects for anticancer therapy in the future.

Immunohistochemical staining for p53

Parallel lines of basic, clinical and epidemiologic research on p53 are now converging and research findings are being translated into medical practice. A big hurdle in applying our understanding of *p53* to the clinics is the sophistication of the molecular techniques required. Molecular-genetic analyses of the p53 gene can be accomplished in several ways (Hall and Lane, 1994). For example, mutations in the *p53* gene can be demonstrated by sequence analysis and allelic deletions can be detected through Loss Of Heterozygosity (LOH) analyses. These molecular-genetic analyses of the *p53* gene are, however, very elaborate and meticulous processes and these analyses are therefore usually confined to exons 5-8. Exons 5-8 contain the sequence specific DNA binding domains of p53. Because mutations outside this region are uncommon, limiting analyses to this region is usually justified (Hollstein et al., 1991). However, even when confined to these exons, molecular-genetic analyses of *p53* are still time consuming and cumbersome procedures, especially on archival material and these techniques are therefore not generally performed in routine pathology laboratories.

A number of investigators have therefore examined alternative methods for determining p53 status. Wild-type p53 protein has a very short half-life and is generally not detectable with immunohistochemical techniques. Mutant p53 gene product, however, is characterized by conformational changes in the protein that prolong its half-life and stability (Finlay et al., 1988). As a result, mutant p53 protein is immunohistochemically detectable, while wild-type p53 is not. For many neoplasms, immunohistochemical staining for the p53 protein is a good substitute for analyzing mutations at the DNA level (van Edkardstein et al., 1997). Furthermore, immunostaining is a standard procedure available in most pathology laboratories and the technique is therefore suitable for application in a clinical setting.

It is, however, important to realize that immunohistochemical staining for the p53 protein is an imperfect indicator of molecular-genetic alterations of the *p53* gene (Wynford-Thomas, 1992; Battifora, 1994; Hall and Lane, 1994). First, technical conditions limit the usefulness of immunohistochemistry as a means of evaluating p53-status. There are several commercially available monoclonal and polyclonal antibodies for immunohistochemical detection of p53 protein. These antibodies all bind to different epitopes of the p53 protein and there are substantial differences in the sensitivity of these antibodies (Baas et al., 1994). A primary antibody with relative low affinity for p53 can cause false-negative results. To select a primary antibody, several antibodies should be tested under the same conditions that will be used to evaluate the clinical samples. Another technical problem is the masking of epitopes, caused by the method of fixation. Routinely formalin-fixed paraffin-embedded tissues show a decreased sensitivity for p53 immunostaining compared to fresh-frozen tissue. This may also account for false-negative results, but epitope masking can be overcome with a variety of recently developed antigen enhancement techniques. These techniques make archival tissue specimens accessible for p53 immunostaining. However, lowering the detection threshold for p53 protein can potentially lead to the detection of wild-type p53 protein and thus to false-positive results (Hall and Lane, 1994). Indeed, antigen enhancement has been shown to give false-positive non-specific staining when combined with certain antibodies against p53 (Baas et al., 1996). Thus, the combination of a specific antibody with a specific antigen enhancement system must be tested before the combination is used to evaluate clinical samples for p53 function.

Second, biological mechanisms can also account for some of the discrepancies between molecular-genetic and immunohistochemical analyses of p53. For example, functional expression of p53 protein can also be found in response to cellular stresses. Irradiation has been shown to produce immunohistochemically detectable levels of wild-type p53 in normal cells (Kuerbitz et al., 1992; Fritsche et al., 1993). Furthermore, some molecular-genetic alterations of the p53 gene abrogate or completely abolish the production of p53 protein. These mutations would result in false-negative immunohistochemistry for the p53 protein, because p53 levels would not reach immunohistochemically detectable levels (Baas et al., 1994). Similarly, some point mutations may not stabilize the p53 protein sufficiently to reach levels detectable by immunohistochemistry (Wynford-Thomas, 1992). With the discovery of *p21*, an effector of functional p53 (Fig. 1) and the subsequent development of immunohistochemical staining techniques for the p21 protein, it seemed that these false-negative results could be overcome. *p21* is transcriptionally activated by functional p53 protein. Therefore, additional evaluation of p21 protein expression could serve as a potential discriminator between true-negative and false-negative immunostaining for the p53 protein. However, we and various other investigators have found that p21 expression can occur independent of functional p53 protein (Slebos et al., 1996). Although p21 expression may still be of value as an indicator of the integrity of the p53 tumor suppressor pathway, its value above and beyond p53 protein expression is questionable.

The first two limitations of immunohistochemistry in detecting p53 dysfunction were technical and biological. A third category limiting its validity is the interpretation of the staining. Patterns of expression of the p53 protein differ substantially and the various expression patterns are subject to individual interpretation (Fisher et al., 1994; Kay et al., 1996). This limits the usefulness of immunohistochemistry in a clinical setting and creates the need for a clear and explicit understanding of how to interpret these different

expression patterns in a uniform manner.

Conversely, immunostaining may be better then genetic analyses in some situations. In most studies, molecular-genetic analysis of the *p53* gene is confined to the evolutionarily conserved hotspots in exons 5-8. However, mutations outside these exons, as well as within intronic sequences, have been reported (Slebos et al., 1998). These mutations would not be detectable by sequencing exons 5-9, but they could lead to increased stability and nuclear accumulation of p53 protein, resulting in positive immunostaining.

There are at least three other situations in which immunohistochemical staining for the p53 protein would more accurately reflect p53 function then would genetic analyses. These include: stabilization of the p53 protein by interaction with another protein; mutations in the *p53* promoter region; and functional overexpression of *p53*. Positive immunohistochemical staining in the first case is indicative of dysfunctional p53 protein and should therefore be considered of value, although strictly speaking, no molecular-genetic alterations underlie the protein overexpression. For example, Cytomegalovirus (Kovacs et al., 1996; Garcia et al., 1997) and Epstein-Barr Virus (Niedobitek et al., 1993) gene products both can stabilize p53 protein after cellular infection. Immunohistochemistry could serve as a valid test for establishing p53 dysfunction in these cases. Similarly, mutations in the *p53* promotor region can also lead to overexpression of wild-type, but dysfunctional p53 (Barnes et al., 1992). The dysfunctional nature of this p53 protein is best illustrated by the cancer-prone phenotype of the family in which this mutation was first described (Barnes et al., 1992). Again, immunohistochemistry would detect p53 dysfunction in these cases, whereas mutational analysis of exons 5-9 of the p53 gene would not.

Immunohistochemistry has one final distinct advantage over molecular-genetic analysis. Immunohistochemistry is performed on tissue sections. Histopathological features can be taken into account when evaluating the staining of cells. Obviously, this morphologic information is lost in molecular-genetic analyses.

Conclusion

In conclusion, the rapid discovery of the various components of the p53 tumor-suppressor pathway offers a new understanding of the pathophysiology of carcinogenesis and should eventually contribute to better patient management. The use of immunohistochemistry to detect p53 dysfunction is restricted, due to limitations of technical and biological nature and because of ambiguities in the interpretation of results. Yet, immunohistochemistry has some distinct advantages over molecular-genetic analyses. Tissue morphology is preserved and the functional mediator of the p53 gene, the p53 protein itself, is evaluated. We conclude that immunohistochemistry for p53 protein can be considered an adjunct of equal importance compared to advanced molecular-genetic analysis of the p53 gene and that these techniques can be used in a complementary fashion. Immunohistochemistry is currently the only technique applicable as a routine screening test for p53 dysfunction. Such a test is particularly valuable for clinical application of the p53 tumor-suppressor pathway. Therefore, eliminating limitations of immunohistochemistry for p53 should be a goal of future research.

References

Baas I.O., Mulder J.W.R., Offerhaus G.J.A., Vogelstein B. and Hamilton, S.R. (1994). An evaluation on six antibodies for immunohistochemistry of mutant *p53* gene product in archival colorectal neoplasms. J. Pathol. 172, 5-12.

Baas I.O., van den Berg F.M., Mulder J.W.R., Clement M.J., Slebos R.J., Hamilton S.R. and Offerhaus G.J. (1996). Potential false-positive results with antigen enhancement for immunohistochemistry of the p53 gene product in colorectal neoplasms. J. Pathol. 178, 264-267.

Baker S.J., Preisinger A.C., Jessup J.M., Paraskeva C., Markowitz S., Willson J.K., Hamilton S. and Vogelstein B. (1990). p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res. 50, 7717-7722.

Barnes D.M., Hanby A.M., Gillett C.E., Mohammed S., Hodgson S., Bobrow L.G., Leigh I.M., Parkis T., MacGeoch C., Spurr N.K. and Lane D.P. (1992). Abnormal expression of wild-type p53 protein in normal cells of a cancer family patient. Lancet 340, 259-263.

Battifora H. (1994). p53 immunohistochemistry: a word of caution. Human Pathol. 25, 435-437.

Clarke A.R., Purdie C.A., Harrison D.J., Morris R., Bird C.C., Hooper M.L. and Wylie A.H. (1993). Thymocyte apoptosis induced by p53 dependent and independent pathways. Nature 362, 849-852.

Dickman S. (1997). First p53 relative may be a new tumor suppressor. Science 277, 1605-1606.

Donehower L.A., Harvey M., Slagle B.L., McArthur M.J., Montgomery C.A. Jr., Butel J.S. and Bradley A. (1992). p53 deficient mice are developmentally normal but susceptible to spontaneous tumors. Nature 356, 215-221.

Dowell S.P. and Hall P.A. (1995). The p53 tumour suppressor gene and tumour prognosis: is there a relationship? J. Pathol. 177, 221-224.

Esrig D., Elmajian D., Groshen S., Freeman J.A., Stein J.P., Chen S.C., Nichols P.W., Skinner D.G., Jones P.A. and Cole R.J. (1994). Accumulation of nuclear p53 and tumor progression in bladder cancer. N. Engl. J. Med. 331, 1259-1264.

Finlay C.A., Hinds P.W., Tan T.H., Eliyahu D., Oren M. and Levine A.J. (1988). Activating mutations for transformation by p53 produce a gene product that forms a hsc700-p53 complex with an altered half-life. Mol. Cell Biol. 8, 531-539.

Fisher C.J., Gillett C.E., Vojtesek B., Barnes D.M. and Millis R.R. (1994). Problems with p53 immunohistochemical staining: the effect of fixation and variation in the methods of evaluation. Br. J. Cancer. 69, 26-31.

Fritsche M., Haessler C. and Brandner G. (1993). Induction of nuclear accumulation of the tumor suppressor protein p53 by DNA damaging agents. Oncogene 8, 307-318.

Garcia J.F., Piris M.A., Lloret E., Orvadrre J.L., Murillo P.G. and Martinez J.C. (1997). p53 expression in CMV-infected cells:

association with the alternative expression of the p53 transactivated genes p21/Waf1 and MDM2. Histopathology 30, 120-125.

Hall P.A. and Lane D.P. (1994). p53 in tumour pathology: can we trust immunohistochemistry?-revisited! J. Pathol. 172, 1-4.

Hall P.A., Meek D. and Lane D.P. (1996). p53-Integrating the complexity. J. Pathol. 180, 1-5.

Hall P.A., Ray A. and Lemoine N.R. (1991). p53 immunostaining as a marker of malignant disease in diagnostic cytopathology. Lancet 338, 513.

Harris C.C. (1996). Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. J. Natl. Cancer Inst. 80, 1442-1455.

Harris C.C. and Hollstein M. (1993). Clinical implications of the p53 tumor-suppressor gene. N. Engl. J. Med. 329, 318-1327.

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

Hung J. and Anderson R. (1997). p53: functions, mutations and sarcomas. Acta Orthop. Scand. Suppl 273, 68-73.

Ireland A.P., Clark G.W.B. and DeMeester T.R. (1997). Barret's esophagus. The significance of p53 in clinical practice. Ann. Surg. 225, 17-30.

Jost C.A., Marin M.C. and Kaelin W.G. Jr. (1997). p73 is a human p53-related protein that can induce apoptosis. Nature 389, 191-194.

Kay E.W., Barry Walsh C.J., Whelan D, O'Grady A. and Leader M.B. (1996). Inter-observer variation of p53 immunohistochemistry - an assessment of a practical problem and comparison with other studies. Br. J. Biomed. Sci. 53,101-107.

Kern S.E. (1994). p53: Tumor suppression through control of the cell cycle. Gastroenterology 106, 1708-1711.

Kern S.E., Kinzler K.W., Bruskin A., Jarosz D., Friedman P., Prives C. and Vogelstein B. (1991). Identification of p53 as a sequence specific DNA-binding protein. Science 252, 1708-1711.

Kinzler K.W. and Vogelstein B. (1994). Clinical implications of basic research. Cancer therapy meets p53. N. Engl. J. Med. 331, 49-50.

Kinzler K.W. and Vogelstein B. (1996). Life (and death) in a malignant tumour. Nature 379,19-20.

Kleihues P., Schauble B., zur Hausen A., Esteve J. and Ohgaki H. (1997). Tumors associated with p53 germline mutations. A synopsis of 91 families. Am. J. Pathol. 150, 1-13.

Knudson A.G. Jr. (1971). Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. USA 68, 820-823.

Kovacs A., Weber M.L., Burns L.J., Jacob H.S. and Vercellotti G.M. (1996). Cytoplasmic sequestration of p53 in cytomegalovirus-infected human endothelial cells. Am. J. Pathol. 149, 1531-1539.

Kuerbitz S.J., Plunkett B.S., Walsh W.V. and Kastan M.B. (1992). Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. USA 89, 7491-7495.

Lane D.P. (1992). p53, guardian of the genome. Nature 358, 15-16.

Levine A.J. (1992). The p53 tumor suppressor gene. N. Engl. J. Med. 326, 1350-1352.

Lowe S.W., Bodis S., McClatchey A., Remington L., Ruley H.E., Fisher D.E., Housman D.E and Jacks T. (1994). p53 status and the efficacy of cancer therapy in vivo. Science 266, 807-810.

Lowe S.W., Ruley H.E., Jacks T. and Housman D.E. (1993). p53 dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 74, 957-967.

Malkin D., Li F.P., Strong L.C., Fraumeni J.F. Jr., Nelson C.E., Kim D.H., Kassel J., Gryka M.A., Bischoff F.Z., Tainsky M.A. and Friend S.H. (1990). Germline p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. Science 250, 1233-1238.

Niedobitek G., Agathanggelou A., Barber P., Smallman L.A., Jones E.L. and Young L.S. (1993). p53 overexpression and Epstein-Barr Virus infection in undifferentiated and squamous cell nasopharyngeal carcinomas. J. Pathol. 170, 457-461.

Nishida N., Fukuda, Y., Ishizaki K. and Nakao K. (1997). Alternations of cell cycle-related genes in hepatocarcinogenesis. Histol. Histopathol. 12, 1019-1025.

Oliner J.D., Kinzler K.W., Meltzer P.S., George D.L. and Vogelstein B. (1992). Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 358, 80-83.

Oren M. (1997). Lonely no more: p53 finds its kin in a tumor suppressor haven. Cell 90, 829-832.

Serth J., Kuczyk M.A., Bokemeyer C., Hervatin C., Nafe R., Tan H.K. and Jonas U. (1995). p53 immunohistochemistry as an independent prognostic factor for superficial transitional cell carcinoma of the bladder. Br. J. Cancer 71, 201-205.

Slebos R.J.C., Baas I.O., Clement M.J., Polak M., Muldar J.W., van den Berg F.M., Hamilton S.R. and Offerhaus G.J. (1996). Clinical and pathological associations with p53 tumor suppressor gene mutations and expression of p21 Waf1/Cip1 in colorectal carcinoma. Br. J. Cancer 74, 165-171.

Slebos R.J.C., Baas I.O., Clement M.J., Offerhaus G.J.A., Askin F.B., Hruban R.H. and Westra W.H. (1998). p53 alterations in atypical alveolar hyperplasia of the human lung. Hum. Pathol. (in press).

Soini Y., Turpeenniemi-Hujanen T., Kamel D., Autio-Harmainen H., Risteli J., Risteli L., Nuorva K., Paako P. and Vahakungas K. (1993). p53 immunohistochemistry in transitional cell carcinoma and dysplasia of the urinary bladder correlates with disease progression. Br. J. Cancer 68, 1029-1035.

Speir E., Modali R., Huang E.S., Leon M.B., Shawl F., Finkel T. and Epstein S.E. (1994). Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. Science 265, 391-394.

Srivastava S., Zou Z.Q., Pirollo K., Blattner W. and Change E.H. (1990). Germline transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature 348, 747-749.

van Eckardstein K., Gries H., Bolik E., Cervos-Navarro J., Tschairkin I.N., and Patt S. (1997). p53 mutation and protein alteration in 50 gliomas. Restrospective study by DNA-sequencing techniques and immunohistochemistry. Histol. Histopathol. 12, 611-616.

Vogelstein B. and Kinzler K.W. (1992). p53 function and dysfunction. Cell 70, 523-526.

Wu X., Bayle J.H., Olson D. and Levine A.J. (1993). The p53-MDM-2 autoregulatory feedback loop. Genes Dev. 7, 1126-1132.

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

Wynford-Thomas D. (1996). p53: Guardian of cellular senescence. J. Pathol. 180, 118-121.

Xiong Y., Hannon G.J., Zhang H., Casso D., Kobayashi R. and Beach D. (1993). p21 is a universal inhibitor of cyclin kinases. Nature 366, 701-704.

Accepted July 27, 1998