

review

p53 - the paradigm of tumor-suppressor genes?

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p53 is a tumor-suppressor gene the alterations of which are among the most frequent genetic changes detected in human neoplasms. Its product - p53 protein is a component of several biochemical pathways that are central to carcinogenesis: DNA transcription, genomic stability, DNA repair, cell cycle control, and apoptosis. The analysis of the spectrum of p53 mutations and insight into the p53 mediated biochemical pathways of programmed cell death and cell cycle arrest, provide clues to the understanding of molecular pathogenesis of cancer and of mechanisms related to p53 mediated tumor suppression. The purpose of the present article is to summarise the most important facts concerning p53 since understanding of the above listed processes might provide the potential molecular targets for the development of a rational cancer treatment.

Key words: neoplasms; genes, suppressor, tumor; genes, p53; protein p53

Historical background

In 1979, Lane and Crawford,¹ as well as Linzer and Levine² independently discovered p53 as a nuclear 53kd phosphoprotein tightly associated with the large T antigen in the SV40 tumor virus-transformed cells. Originally, p53 protein came to be classified as a tumor antigen since it was suggested that the interaction of p53 with the large T antigen was important for transformation.^{1,2} The p53 cDNA constructs isolated in this period were all derived from tumor cells³ and were found to cooperate with the ras oncogene to transform rat fibroblasts in cell culture.^{4,5} So, p53 came to be classified as an oncogene. Finally,

in late 1980s, all the transforming p53 cDNA clones were discovered to be mutant forms of p53, while the wild-type gene isolated from normal cells failed to induce neoplastic transformation and even inhibited tumor cell growth or blocked the neoplastic transformation.⁶⁻¹⁰ Now p53 looks like being a tumor-suppressor gene, negatively regulating the cell cycle and requiring loss-of-function mutations for tumor formation. However, unlike other classical tumor-suppressor genes, at least some mutated p53 forms act as dominant transforming oncogenes.¹¹

Structure and regulation

The p53 gene spans a moderately-sized segment of DNA, located on the short arm (17p13) of chromosome 17 and is ultimately

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translated to a phosphoprotein consisting of 393 amino acids contained in 11 exons, the first of which is noncoding.¹² Five evolutionary conserved domains within the coding regions are supposed to be essential to the functional activity of p53.^{12,13}

The N-terminal domain (residues 1-42) interacts with the subunits of the general transcription factors TFIID and TFIIF and acts as a transcriptional activator. This domain also binds the MDM-2 protein - a negative regulator of p53, and adenovirus E1B protein. The core domain (residues 100-300) harbors the sequence specific dsDNA binding function of p53, and encodes the binding site for SV40 large T antigen and, possibly, for the papillomavirus E6 protein. The C terminal domain (residues 300-393) has multiple functional activities, including nonspecific DNA binding and reannealing of complementary ssDNA oligonucleotides. Residues 320 to 355 are involved in oligomerization, and the very terminal C domain (residues 360-393) binds ssDNA ends and regulates specific DNA binding by the core domain.¹⁴⁻¹⁸

It appears that p53 alone assembles into inactive forms and requires activating factors to confer an effective sequence-specific DNA binding capacity. Such a regulation is exerted by the C-terminal end of p53 itself. Hupp *et al.* proposed a model according to which the C-terminus negatively regulated specific DNA binding by interacting with a region in another p53 molecule within the tetramer.¹⁹ This locked the tetramer in a conformation that was incapable of specific DNA binding.

p53 contains multiple phosphorylation sites located at both the C and N-termini of the molecule. Eight different protein kinases are involved in p53 phosphorylation: p34cdc2 kinase, DNA-activated protein kinase, mitogen activated protein kinases, protein kinase C, casein kinase I and II, Raf-1 kinase, and Jun kinase.²⁰⁻²⁶ p34cdc2 kinase (an A- and B-cyclin dependent kinase) phos-

phorylates at serine 315 and thus stimulates the specific binding of DNA to the consensus sequence of p53 and also causes a specific conformational change of the protein.²⁰ DNA-activated protein kinase and mitogen activated protein kinases are involved in the phosphorylation of p53 at the N terminal domain, influencing the transcriptional activity and the half-life of the protein.^{21,22} Protein kinase C-dependent, direct or indirect, phosphorylation of serine residues 372-381 at the C terminal of the p53 tetramer is a critical event for the transition from the latent to the active form of p53. Namely, the "open" configuration of the four phosphorylated C ends of the tetramer is a necessary prerequisite for the nonspecific DNA binding which, in turn, allows the consequent specific DNA binding to p53 consensus motifs.^{19,23,27} The phosphorylation of the serine 392 is dependent on casein kinase II, however, this site is less critical for p53 activation.²⁸

Another form of p53 regulation is exerted at the level of p53 protein stability. In normal cells, p53 shows a relatively short half-life (about 20 minutes) due to its rapid turnover, yet its half-life can be extended to hours following some kinds of cellular stress or as a consequence of mutations involving the core domain. Stability of the protein is affected by its complex formation with a number of cell proteins that are capable of slowing-down or preventing its ubiquitin-pathway degradation.^{29,30}

It is still uncertain which physiological signal activates p53 after an appropriate stimulus. Possible candidates are p300 and the closely related transcription factor CBP that bind to N-terminal domain of p53. p300 acetylates conserved lysine residues in the p53 C-terminal domain which results in the activation of specific DNA binding of p53.^{31,32}

Intracellular localisation, concentration, and state of phosphorylation of p53 are cell-cycle dependent. Activity of wild-type p53

protein demands nuclear localisation of the protein which occurs close to the beginning of S phase. Following the beginning of DNA synthesis, p53 accumulates again in the cytoplasm.^{33,34}

Function

p53 protein is implicated in nearly all forms of cell growth stimulation and inhibition. It may be required early in the induction of cell proliferation and is also a transcriptional regulatory protein, capable of both stimulating and repressing gene expression.³⁵ p53 binds DNA in a sequence-specific manner and also influences gene expression indirectly by interacting with other transcription factors.^{35,36} In certain cell types, over-expression of p53 induces apoptosis.^{23,37,38} p53 may regulate in vitro cellular senescence and, under the influence of certain cytokines, it cooperates in the induction of differentiation.^{39,40}

Transcription dependent pathway

Several genes were found to be transcriptionally activated by p53, including MDM-2, p21, GADD45, cyclin G1, BAX, FAS, transforming growth factor- α , muscle creatinine kinase, and insulin-like growth factor-binding protein 3.^{10,41-48}

Following DNA damage, p53 protein rapidly accumulates in the nucleus. The C-terminal domain of p53 recognises the damaged DNA, and the accumulation of p53 is probably a consequence of conformational change of the protein which leads to reduced degradation by ubiquitin degradation pathway or, less likely, a consequence of increased synthesis of p53 protein.^{33,38,49} At the same time, no changes in p53 mRNA levels are observed.⁵⁰

The p53 protein, in turn, activates downstream genes whose products are involved in growth inhibition, e.g. p21 and GADD45.

p21 is a cyclin-dependent kinase inhibitor that inhibits the activity of cyclin D-cdk 4/6 causing a hypophosphorylation of retinoblastoma protein (Rb), thus preventing the release of E2F and blocking the G1-S transition.^{51,52} Transactivation of GADD45, the protein product of which binds proliferating cell nuclear antigen and inhibits S phase entry, may contribute to the p53 dependent cell cycle arrest pathway.⁵³ Insulin-like growth factor-binding protein 3 gene which encodes a protein that binds insulin-like growth factor and thus inhibits its growth signalling is another p53 target gene that may function in this pathway.⁴⁸ p53 also regulates the G2/M checkpoint of the cell cycle, yet the mechanism of p53 mediated G2/M control is unknown.^{54,55}

The expression of MDM-2 protein is regulated by the level of wild-type p53 protein. The MDM-2 protein, in turn, forms a complex with p53 and decreases its ability to act as a positive transcription factor - which represents a negative feedback loop to buffer changes in p53 levels.^{56,57}

Transcription independent pathway

Modulation of cellular processes goes often via the mechanism of protein-protein interactions. In agreement with its multifunctional qualities, p53 protein associates with a group of viral and cellular proteins that may play an important role in the p53 mediated and transcription independent pathway (Table 1).^{35,36}

Several basic transcription factors, including TATA binding protein, TATA binding protein-associated proteins, TFIIF-associated factor p62 form a complex with p53.⁵⁸⁻⁶¹ Binding of TATA binding protein to p53 protein has been implicated to be responsible for p53 mediated transcriptional repression. The list of genes reported to be transrepressed by p53 consists of proliferating cell nuclear antigen, interleukin 6, Rb gene, multidrug-resistance (MDR) gene, p53, BCL-2, inducible

Table 1. Some of viral and celllural proteins that associate with p53

Viral proteins	Cellular proteins
human papilloma virus E6	heat-shock protein 70
simian virus 40 T antigen	MDM-2
Epstein-Barr nuclear antigen	ubiquitin-ligase E6-AP
adenovirus E1B	transcription factor WT-1

nitric oxide synthase-2.⁶²⁻⁶⁷ The binding of p53 to replication protein A also alludes to the possible direct role of p53 in DNA replication and nucleotide excision repair.⁶⁸

p53 in the nucleotide excision DNA repair

The observations that p53 can selectively bind to several DNA helicases, including XPB and XPD, which are a part of transcription factor TFIIH, led to the hypothesis that p53 may play a direct role in modulating DNA nucleotide excision repair.^{69,70} Furthermore, p53 can also recognise several forms of damaged DNA (mismatched DNA, ssDNA ends).⁷¹ So, a new model emerged in which p53 may act as a sensor that binds to damaged parts and recruits the nucleotide excision repair machinery by trapping TFIIH (i.e. the major component of the repair complex) at regions where it is needed which, in turn, facilitates the constitution of a functional "repairosome".⁷²

p53 mediated apoptosis

The molecular mechanisms behind p53 induced apoptosis are only partially explained. The current idea is that DNA damage induces stabilization of the p53 protein which promotes DNA repair by assembling the repair machinery.^{70,71} In case the DNA damage is unreparable, p53 triggers cells to undergo apoptotic death to prevent propagation of the cells carrying a mutation. Several activities of p53 have been identified that could participate in the process of programmed cell death. Namely, p53 upregulates

the expression of BAX and downregulates expression of BCL-2, all of which have been implicated in modulation of apoptosis.⁷³ Another possible explanation for the induction of apoptosis could be that the transactivation of insulin-like growth factor-binding protein 3 gene and thus increased insulin-like growth factor-binding protein 3 levels may presumably block an insulin-like growth factor mediated survival signal and lead to apoptosis.⁴⁸ Finally, a whole series of new p53 induced genes related to redox control have been discovered that lead to the formation of reactive oxygen species, oxidative degradation of mitochondrial components and apoptotic cell death.⁷⁴ Beyond this, a transactivation independent function of p53 in the triggering of the apoptotic pathway has been implicated and may well be performed by a proline rich region located between residues 64 and 91 in p53 molecule. The proline rich region may provide a crucial accessory apoptotic signal, perhaps by interacting with a cellular SH3-domain-containing partner protein.^{75,76}

Briefly, the major role of p53 is being a monitor of cellular proliferation (guardian of the genome) and a determinant of response to DNA damage.

Mutations

The p53 mutations are found in the preponderance of human tumors and the functional p53 is lost in approximately half of all human malignancies.^{52,77}

The majority of p53 mutations are mis-sense point mutations giving rise to single

amino acid substitutions that abrogate the specific DNA binding activity.¹⁸ Concomitantly, the half-life of p53 extends from normal 20 minutes (wild-type protein) to approximately 48 hours (mutant protein) resulting in nuclear accumulation of the mutant protein.^{78,79}

Most of the mutations are clustered in the most highly conserved domains of the gene spanned by four to nine exons. There are at least three mutation "hot spots" affecting the residues 175, 248, and 273.⁸⁰ Although mutations of the p53 gene are most frequently acquired, they can also be inherited through the Li-Fraumeni syndrome. In these families, one mutant p53 allele is inherited, and the second allele acquires a mutation.⁸¹

p53 is not inactivated only through mutation, but also at the protein level through complexing with DNA tumor viral oncoproteins like the SV40 large T antigen, the adenovirus E1B protein, and the human papilloma virus E6 protein⁸² or cellular protein MDM-2.⁵⁷

Detection of p53 mutations

In respect to the fact that inactivation of p53 in tumor cells leads to the increased cellular proliferation and inhibition of apoptosis and concerning the observations that mutations of p53 gene are associated with advanced disease, poor response to chemotherapy or radiotherapy, and short survival,^{83,84} it is of great importance to determine the p53 status in every patient prior to treatment. Various approaches to the detection of p53 mutations have evolved in the last 19 years and each of them has certain advantages and certain disadvantages.^{77,85}

The most informative method for the study of p53 mutations is the determination of the nucleotide sequence with either direct sequencing or indirect molecular analysis.⁸⁵ Molecular sequencing is the only way to eval-

uate the mutational event that inactivates the gene (there is no accumulation of the mutant protein) and allows for the unequivocal detection of alterations. On the other hand, the indirect molecular methods as denaturing gradient electrophoresis, single-strand conformational polymorphism analysis or variants as hydroxylamine and osmium tetroxide chemical cleavage, and pulse field gel electrophoresis, are more suitable for screening and easier to perform. Yet, both methods share some drawbacks - namely, they cannot be at the moment performed in routine diagnosis, tumor tissue is required, and care must be taken to avoid contamination from an excess of normal tissue.⁸⁵⁻⁸⁷

Immunohistochemical and immunocytochemical methods, under optimum conditions, are capable of detecting most missense mutations (which result in nuclear accumulation of the mutant protein) and can also identify p53 stabilization without mutations (a consequence of the alteration of pathways regulating p53 expression). On the contrary, the mutations which do not induce p53 overexpression (nonsense mutations, frame-shift mutations, splice mutations, gene deletions, promoter mutations) will go undetected. Immunohistochemical results can be affected by the degradation of antigen during tissue processing and by the specificity of the antibodies used. Tumor tissue is needed, but the contamination by normal tissues is not a critical factor. In sum, p53 immunostaining is still an imperfect reflection of the prevalence of p53 mutations.^{85,86,88-90}

It remains controversial whether the mutant p53 protein can be detected in patients sera, since the results of various determinations are opposing. Namely, two groups of authors determined the serum levels of mutant p53 in patients with malignant lymphomas using a commercially available ELISA kit,^{91,92} while another group of authors failed to do so using the same ELISA method in patients with lung cancer.⁸⁷ Simi-

larly, Hassapoglidou using immunofluorimetric method could not detect mutated p53 protein in sera of patients with cancer.⁹³

Although p53 is a cellularly encoded protein, it has been found to be immunogenic and capable of eliciting a p53 specific antibody immune response. About one third of patients (the percentage varies for different types of cancer) with tumors that carry p53 missense mutations develop circulating p53 antibodies. These antibodies are not seen if there is no p53 accumulation in the tumor cell and, in case of lung carcinoma, they can appear before the cancer is detectable. The p53 protein may either be released during tumor cell necrosis, or otherwise translocates to the surface of the cell, inducing a B-cell response as a result of the breakdown of the immune system tolerance. Methods used for the determination of p53 antibodies include ELISA, immunoblot, and immunoprecipitation techniques. These methods can be performed routinely, they do not require tumor tissue, and can be used for follow up. Therefore, assessment of serum p53 antibodies is quite specific, but has low sensitivity (some mutations do not induce the production of p53 antibodies) in the detection of p53 mutations.^{85,87,94-99}

Therapeutic approaches

Several therapeutic approaches are currently being assessed against the growth advantage and resistance to chemotherapy and radiotherapy observed in tumor cells with p53 mutations. The first approach is the investigation of active immunization against the potential tumor antigens carried by mutated p53, and indeed, it has been shown that it is possible to generate p53 specific CD8+ cytotoxic T lymphocytes by immunizing mice with mutated p53 protein.¹⁰⁰ Furthermore, it was observed that a monoclonal antibody to p53, PAb 421, and a small peptide derived

from p53 (the C-terminal domain) are able to restore the sequence specific DNA binding as well as growth suppression function of at least some mutant p53 proteins (by inducing a change in the configuration with a return to the active wild-type configuration).^{19,101-103}

Among the recently proposed approaches, two are quite interesting. The first uses an adenovirus defective for E1B gene, which replicates only in the cells lacking functional p53 but not in the cells with wild-type p53, leading to selective destruction of tumor cells with mutant p53.¹⁰⁴ The second, on the other hand, utilizes the transfer of a cytotoxic gene which is only activated in the presence of a mutant p53, resulting in a selective killing of tumor cells with p53 mutation.¹⁰⁵

However, the most promising approach is p53 gene transfer in tumor cells carrying a p53 mutation. In tumor cells lacking functional p53, such a transfer can lead to tumor regression, as well as improve the cytotoxicity of antineoplastic agents, and the response to ionizing radiation.¹⁰⁶⁻¹⁰⁸ The most frequently used vectors for p53 gene transfer in animal models have been recombinant adenoviruses, and less often retroviruses, which have a lower capacity of gene transfer *in vivo*. Interestingly, some tumor regressions were more important than expected. They were indicated by the percentage of p53 transfected cells, suggesting a possible "bystander" effect, with destruction of non-transfected cells in the vicinity of transfected cells, as for suicide gene transfer.¹⁰⁹

And finally, another idea was to try to identify the drugs that may trigger programmed cell death through a p53 independent pathway. It has been suggested that taxol could be one of them, however, the clinical results with taxol were poorer in patients with mutant p53.^{110,111}

Latest findings

Even though p53 seems to play a central role in nearly all forms of cell growth stimulation and inhibition and was termed as the "guardian of the genome", it is becoming obvious that other proteins, as for example the recently discovered p33 and p75, also take an important part in the regulative mosaic.

The nuclear protein p33 (a product of the tumor-suppressor gene ING1) forms a complex with p53 and cooperates in the negative regulation of cell proliferation by modulating p53 dependent transcriptional activation.^{112,113}

p73 is a protein that is closely related to p53, both structurally and functionally; however, it is induced by different signals and thus plays a fundamentally different role in the maintenance of cell homeostasis. It can, at least when overproduced, activate p53 responsive genes and act as a growth suppressor.^{114,115}

References

- Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. *Nature* 1979; **278**: 261-3.
- Linzer DJ, Levine AJ. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* 1979; **17**: 43-52.
- Jenkins JR, Rudge K, Currie GA. Cellular immortalization by a cDNA clone encoding the transformation-associates phosphoprotein p53. *Nature* 1984; **312**: 651-4.
- Eliyahu D, Raz A, Gruss P, Givol D, Oren M. Participation of p53 cellular tumor antigen in transformation of normal embryonic cells. *Nature* 1984; **312**: 646-9.
- Parada LF, Land H, Weinberg RA, Wolf D, Rotter V. Cooperation between gene encoding p53 tumor antigen and ras in cellular transformation. *Nature* 1984; **312**: 649-51.
- Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. Activatin mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. *Mol Cell Biol* 1988; **8**: 531-9.
- Eliyahu D, Goldfinger N, Pinhasi-Kimhi O, Shaulsky G, Skurnik Y, Arai N, et al. Meth A fibrosarcoma cells express two transforming mutant p53 species. *Oncogene* 1988; **3**: 313-21.
- Hinds PW, Finlay CA, Levine AJ. Mutation is required to activate the p53 gene for cooperation with the ras oncogene and transformation. *J Virol* 1989; **63**: 739-46.
- Finlay CA, Hinds PW, Levine AJ. The p53 protooncogene can act as a suppressor of transformation. *Cell* 1989; **57**: 1083-93.
- Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B. Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science* 1990; **249**: 912-5.
- Deppert W. The yin and yang of p53 in cellular proliferation. *Cancer Biol* 1994; **5**: 187-202.
- Levine AJ. The p53 tumor suppressor gene and product. *Cancer Surv* 1992; **12**: 59-79.
- Evans HJ. Molecular genetic aspects of human cancers: The 1993 Frank Rose Lecture. *Br J Cancer* 1993; **68**: 1051-60.
- Hinds PW, Weinberg RA. Tumor suppressor genes. *Curr Opin Genet Develop* 1994; **4**: 135-41.
- Meek DW. Post-translational modification of p53. *Semin Cancer Biol* 1994; **5**: 203-10.
- Wang WY, Reed M, Wang P. p53 domains: identification and characterization of two autonomous DNA-binding regimens. *Genes Develop* 1993; **7**: 2575-86.
- Bargonetti J, Manfredi JJ, Chen X, Marshak DR, Prives C. A proteolytic fragment from the central region of p53 has marked sequence-specific DNA-binding activity when generated from wild-type but not from oncogenic mutant p53 protein. *Genes Develop* 1993; **7**: 2565-74.
- Ko LJ, Prives C. p53: puzzle and paradigm. *Genes Develop* 1996; **10**: 1054-72.
- Hupp TR, Sparks A, Lane DP. Small peptides activate the latent sequence specific DNA binding function of p53. *Cell* 1995; **83**: 237-45.
- Bishoff JR, Friedman PN, Marsak C, Prives C, Beach D. Human p53 is phosphorylated by p60-

- cdc2 and cyclin B cdc2. *Proc Natl Acad Sci USA* 1990; **87**: 4766-71.
21. Lees Miller SP, Sakaguki K, Ullrich SJ, Apella E, Anderson CW. Human DNA activated protein kinase phosphorylates serines 15 and 17 in the aminoterminal transactivation domain of human p53. *Mol Cell Biol* 1992; **12**: 5041-6.
 22. Milne DM, Campbell DG, Caudwell FD, Meek DW. Phosphorylation of tumor suppressor protein p53 by mitogen activated protein kinases. *J Biol Chem* 1994; **269**: 9253-60.
 23. Hupp TR, Meek DW, Midgley CA, Lane DP. Regulation of the specific DNA binding function of p53. *Cell* 1992; **71**: 875-86.
 24. Milne DM, Palmer RH, Meek DW. Mutation of casein kinase II phosphorylation site abolishes the antiproliferative activity of p53. *Nucleic Acids Res* 1992; **20**: 5560-70.
 25. Jamal S, Ziff EB. Raf phosphorylates p53 in vitro and potentiates p53 transcriptional transactivation in vivo. *Oncogene* 1995; **10**: 2095-101.
 26. Milne DM, Campbell LE, Campbell DG, Meek DW. p53 is phosphorylated in vitro and in vivo by the ultraviolet radiation-induced protein kinase characteristic of the c-Jun kinase JNK1. *J Biol Chem* 1995; **270**: 5511-8.
 27. Takenaka I, Morin F, Seizinger BR, Kley N. Regulation of the sequence-specific DNA binding function by protein kinase C and protein phosphatases. *J Biol Chem* 1995; **270**: 5405-11.
 28. Fishella M, Zambrano N, Ullrich SG, Lin D, Cho B, Mercer E, et al. The carboxyl-terminal serine 392 phosphorylation site of human p53 is not required for wild type activities. *Oncogene* 1994; **9**: 3249-52.
 29. Tominaga O, Hamelin R, Remvikos Y, Salmon RJ, Thomas G. p53 from basic research to clinical applications *Crit Rev Oncogenesis* 1992; **3**: 257-82.
 30. Shay JW, Werbin H, Funk WD, Wright WE. Cellular and molecular advances in elucidating p53 function. *Mutation Res* 1992; **277**: 163-71
 31. Lill NL, Grossman SR, Ginsberg D, DeCaprio J, Livingston DM. Binding and modulation of p53 by p300/CBP coactivators. *Nature* 1997; **387**: 823-7.
 32. Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* 1997; **90**: 595-606.
 33. Fritsche M, Haessler C, Brandner G. Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene* 1993; **8**: 307-18.
 34. Shaulsky G, Golfinger N, Tosky MS, Levine AJ, Rotter V. Nuclear localization is essential for the activity of p53 protein. *Oncogene* 1991; **6**: 2055-65.
 35. Lane DP. On the expression of the p53 protein in human cancers. *Mol Biol Rep* 1994; **19**: 23-9.
 36. Stuerzbecher HW, Deppert W. The tumor suppressor protein p53: Relationship of structure to function. *Oncol Rep* 1994; **1**: 301-7.
 37. Hoffman B, Lieberman DA. Molecular controls of apoptosis: differentiation/growth arrest primary response genes, proto-oncogenes, and tumor suppressor genes as positive and negative modulators. *Oncogene* 1994; **9**: 1807-12.
 38. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* 1991; **51**: 6304-11.
 39. Shay JW, Pereira-Smith OM, Wright WE. A role for both Rb and p53 in the regulation of human cellular senescence. *Exp Cell Res* 1991; **196**: 33-9.
 40. Spandau DF. Distinct conformations of p53 are observed at different stages of keratinocyte differentiation. *Oncogene* 1994; **9**: 1861-8.
 41. Barak Y, Juven T, Haffner R, Oren M. mdm 2 expression is induced by wild type p53 activity. *EMBO J* 1993; **12**: 461-8.
 42. Kastan MB, Zhan Q, El-Deiry WS, Carrier F, Jacks T, Walsh WV, et al. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in Ataxia-Teleangiectasia. *Cell* 1992; **71**: 587-97.
 43. Okamoto K, Beach D. Cyclin G is a transcriptional target of the p53 tumor suppression protein. *EMBO J* 1994; **13**: 4816-22.
 44. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 1995; **80**: 293-9.
 45. Owen-Schaub LB, Zhang W, Cusack JC, Angelo LS, Santee SM, Fujiwara T, et al. Wild-type human p53 and temperature-sensitive mutant induce Fas/Apo-1 expression. *Mol Cell Biol* 1995; **15**: 3032-40.
 46. Shin TH, Paterson AJ, Kudlow JE. p53 stimulates transcription from the human transforming growth factor alpha promoter: A potential growth-stimulatory role for p53. *Mol Cell Biol* 1995; **15**: 4694-701.

47. Weintraub H, Hauschka S, Tapscott SJ. The MCK enhancer contains a p53 responsive element. *Proc Natl Acad Sci USA* 1991; **88**: 4570-1.
48. Buckbinder L, Talbott R, Velasco-Miguel S, Take-naka I, Faha B, Seizinger BR, et al. Induction of the growth inhibitor IGF-binding protein 3 by p53. *Nature* 1995; **377**: 646-9.
49. Reed M, Woelker B, Wang P, Wang Y, Anderson ME, Tegtmeier P. The C-terminal domain of p53 recognizes DNA damaged by ionizing radiation. *Proc Natl Acad Sci USA* 1995; **92**: 9455-9.
50. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild type p53 is a cell cycle checkpoint determinant following irradiation. *Proc Natl Acad Sci USA* 1992; **89**: 7481-95.
51. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, et al. WAF1, a potential mediator of p53 tumor suppression. *Cell* 1993; **75**: 817-25.
52. Agarwal ML, Taylor WR, Chernov MV, Chernova OB, Stark GR. The p53 network. *J Biol Chem* 1998; **273**: 1-4.
53. Smith ML, Chen IT, Zhan Q, Bae I, Chen CY, Gilmer TM, et al. Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science* 1994; **266**: 1376-80.
54. Stewart N, Hicks GG, Paraskevas F, Mowat M. Evidence for a second cell cycle block at G2/M by p53. *Oncogene* 1995; **10**: 109-15.
55. Guillof C, Rosselli F, Krishnajar K, Moustacchi E, Hoffman B, Lierbermann DA. p53 involvement in control of G2 exit of the cell cycle: Role in DNA damage-induced apoptosis. *Oncogene* 1995; **10**: 2263-70.
56. Wu X, Bayle JH, Olson DC, Levine AJ. The p53-mdm-2 autoregulatory feedback loop. *Genes Develop* 1993; **7**: 1126-32.
57. Momand J, Zambetti GP, Olson DC, George DL, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 1992; **69**: 1237-45.
58. Seto E, Usheva A, Zambetti GP, Momand J, Horikoshi N, Weinmann R, et al. Wild-type p53 binds to the TATA-binding protein and represses transcription. *Proc Natl Acad Sci USA* 1992; **89**: 12028-32.
59. Chen X, Farmer G, Zhu H, Prywes R, Prywes C. Cooperative DNA binding of p53 with TFIID (TBP): A possible mechanism for transcriptional activation. *Genes Develop* 1993; **7**: 1837-49.
60. Thut CJ, Chen JL, Klemm R, Tjian R. p53 transcriptional activation mediated by coactivators TAFii40 and TAFii60. *Science* 1995; **267**: 100-4.
61. Xiao H, Pearson A, Coulombe B, Truant R, Zhang S, Regier JL, et al. Binding of basal transcription factor TFIID to the acidic activation domains of VP16 and p53. *Mol Cell Biol* 1994; **14**: 7013-24.
62. Ginsberg D, Mechta F, Yaniv M, Oren M. Wild-type p53 can down-modulate the activity of various promoters. *Proc Natl Acad Sci USA* 1991; **88**: 9979-83.
63. Santhanam U, Ray A, Sehgal PB. Repression of the interleukin 6 gene promoter by p53 and the retinoblastoma susceptibility gene product. *Proc Natl Acad Sci USA* 1991; **88**: 7605-9.
64. Chin KV, Ueda K, Pastan I, Gottesman MM. Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. *Science* 1992; **255**: 459-62.
65. Deffie A, Wu H, Reinke V, Lozano G. The tumor suppressor p53 regulates its own transcription. *Mol Cell Biol* 1993; **13**: 3415-23.
66. Miyashita T, Harigai M, Hanada M, Reed JC. Identification of a p53-dependent negative response element in the bcl-2 gene. *Cancer Res* 1994; **54**: 3131-5.
67. Forrester K, Ambs S, Lupold SE, Kapust RB, Spillare EA, Weinberg WC, et al. Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase (NOS2) expression by wild-type p53. *Proc Natl Acad Sci USA* 1996; **93**: 2442-7.
68. Dutta A, Ruppert JM, Aster JC, Winchester E. Inhibition of DNA replication Factor RPA by p53. *Nature* 1993; **365**: 79-82.
69. Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepatitis B virus X protein inhibits p53 sequence specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Natl Acad Sci USA* 1994; **91**: 2230-4.
70. Wang XW, Yeh H, Shaeffer L, Roy R, Moncollin V, Egly JM, et al. p53 modulation of THIF-associated nucleotide excision repair activity. *Nature Genet* 1995; **10**: 188-95.
71. Lee S, Elenbase B, Levine AJ, Griffith J. p53 and its 14kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. *Cell* 1995; **81**: 1013-20.
72. Bakalkin G, Yakovleva T, Selivanova G, Magnus-

- son KP, Szekely L, Kiseleva E, et al. p53 binds single-stranded DNA ends and catalyzes DNA renaturation and strand transfer. *Proc Natl Acad Sci USA* 1994; **91**: 413-7.
73. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, et al. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene* 1994; **9**: 1799-805.
74. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. *Nature* 1997; **389**: 300-5.
75. Walker KK, Levine AJ. Identification of a novel p53 functional domain that is necessary for efficient growth suppression. *Proc Natl Acad Sci USA* 1996; **93**: 15335-40.
76. Sakamuro D, Sabbatini P, White E, Prendergast GC. The polyproline region of p53 is required to activate apoptosis but not growth arrest. *Oncogene* 1997; **15**: 887-98.
77. Caron de Fromentel C, Soussi T. TP53 tumor suppressor gene: a model for investigating human mutagenesis. *Genes Chromosom Cancer* 1992; **4**: 1-15.
78. Gannon JV, Greaves R, Iggo R, Lane DP. Activating mutations in p53 produce a common conformational effect: a monoclonal antibody specific for the mutant form. *EMBO J* 1990; **9**: 1595-602.
79. Bartek J, Bartkova J, Vojtesek B, et al. Aberrant expression of the p53 oncoprotein is a common feature of a wide spectrum of human malignancies. *Oncogene* 1991; **6**: 1699-703.
80. Pavletich NP, Chambers KA, Pabo CO. The DNA-binding domain of p53 contains the four conserved regions and the major mutation hot spots. *Genes Develop* 1993; **7**: 2556-64.
81. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. *New Eng J Med* 1993; **329**: 1318-27.
82. Selivanova G, Wiman KG. p53: A cell cycle regulator activated by DNA damage. *Adv Cancer Res* 1995; **66**: 143-80.
83. Visakorpi T, Kallioniemi OP, Heikkinen A, Koivula T, Isola J. Small subgroup of aggressive, highly proliferative prostatic carcinomas defined by p53 accumulation. *J Natl Cancer Inst* 1992; **84**: 883-7.
84. Silvestrini R, Benini E, Daidone MG. p53 as an independent prognostic marker in lymph node-negative breast cancer patients. *J Natl Cancer Inst* 1993; **95**: 965-70.
85. Soussi T, Legros Y, Lubin R, Ory K, Schlichtholz B. Multifactorial analysis of p53 alteration in human cancer: a review. *Int J Cancer* 1994; **57**: 1-9.
86. Wynford-Thomas D. p53 in tumor pathology: Can we trust immunohistochemistry? *J Pathol* 1992; **166**: 329-30.
87. Winter SF, Minna JD, Johnson BE, Takahashi T, Gazdar AF, Carbone DP. Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation. *Cancer Res* 1992; **52**: 4168-74.
88. Hall PA, Lane DP. p53 in tumor pathology: Can we trust immunohistochemistry? - Revisited. *J Pathol* 1994; **172**: 1-4.
89. Battifora H. p53 immunohistochemistry: a word of caution. *Hum Pathol* 1994; **25**: 435-7.
90. Legros Y, Lacabanne V, d'Agay MF, Larsen CJ, Pia M, Soussi T. Production of human p53 monoclonal antibodies and their use in immunohistochemical studies of tumor cells. *Bull Cancer* 1993; **80**: 102-10.
91. Lehtinen T, Aine R, Kellokumpu-Lehtinen P, Hakala T, Lehtinen M. Evaluation of plasma levels of thymidine kinase and mutated p53 in 81 patients with newly diagnosed malignant lymphoma. *Acta Oncol* 1993; **32**: 779-81.
92. Lähdeaho M-L, Lehtinen T, Aine R, Hakala T, Lehtinen M. Antibody response to adenovirus E1b-derived synthetic peptides and serum levels of p53 in patients with gastrointestinal and other malignant lymphomas. *J Med Virol* 1994; **43**: 393-6.
93. Hassapoglidou S, Diamandis EP, Sutherland DJA. Quantification of p53 protein in tumor cell lines, breast tissue extracts and serum with time-resolved immunofluorometry. *Oncogene* 1993; **8**: 1501-9.
94. Schlichtholz B, Legros Y, Gillet D, Gaillard C, Marty M, Lane D, et al. The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. *Cancer Res* 1992; **52**: 6380-4.
95. Trivers GE, DeBenedetti VMG, Cawley HL, Caron G, Harrington AM, Bennett WP, et al. Anti-p53 antibodies in sera from patients with chronic obstructive pulmonary disease can predate a diagnosis of cancer. *Clin Cancer Res* 1996; **2**: 1767-75.
96. Lubin R, Schlichtholz B, Bengoufa D, Zalzman G, Tredaniel J, Hirsch A, et al. Analysis of p53 antibodies in patients with various cancers define B-cell epitopes on human p53: distribution on pri-

- mary structure and exposure on protein surface. *Cancer Res* 1993; **53**: 5872-6.
97. Volkman M, Müller M, Hofmann WJ, Meyer M, Hagelstein J, Raeth U, et al. The humoral immune response to p53 in patients with hepatocellular carcinoma is specific for malignancy and independent of the α -fetoprotein status. *Hepatology* 1993; **18**: 559-65.
98. Caron de Fromentel C, May-Levin F, Mouriesse H, Lemerle J, Chandrasekaran K, May P. Presence of circulating antibodies against cellular protein p53 in a notable proportion of children with B-cell lymphoma. *Int J Cancer* 1987; **39**: 185-9.
99. Angelopoulou K, Diamandis EP, Sutherland DJA, Kellen JA, Bunting PS. Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers. *Int J Cancer* 1994; **58**: 480-7.
100. Yanuck M, Carbone DP, Pendleton CD, Tsukui T, Winter SF, Minna JD, et al. A mutant p53 tumor suppressor protein is a target for peptide-induced CD8⁺ cytotoxic T-cells. *Cancer Res* 1993; **53**: 3257-61.
101. Abarzua P, LoSardo JE, Gubler ML, Neri A. Microinjection of monoclonal antibody PAb421 into human SW480 colorectal carcinoma cells restores the transcription activation function to mutant p53. *Cancer Res* 1995; **55**: 3490-4.
102. Wiczotek AM, Waterman JLP, Waterman MJP, Halazonetis TD. Structure-based rescue of common tumor-derived p53 mutants. *Nature Med* 1996; **2**: 1143-6.
103. Selivanova G, Iotsova V, Okan I, Fritsche M, Stroem M, Groner B, et al. Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. *Nature Med* 1997; **3**: 632-8.
104. Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, et al. An adenovirus mutant that replicates selectively in p53 deficient human tumor cells. *Science* 1996; **274**: 373-6.
105. DeCosta LT, Jen J, He TC, Chan TA, Kinzler KW, Vogelstein B. Converting cancer genes into killer genes. *Proc Natl Acad Sci USA* 1996; **93**: 4192-6.
106. Takahashi T, Carbone D, Takahashi T, Nau MM, Hida T, Linnoila I, et al. Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. *Cancer Res* 1992; **52**: 2340-3.
107. Yang B, Eshleman JR, Berger NA, Markowitz SD. Wild-type p53 protein potentiates cytotoxicity of therapeutic agents in human colon cancer cells. *Clin Cancer Res* 1996; **2**: 1649-57.
108. Spitz FR, Nguyen D, Skibber JM, Meyn RE, Cristiano RJ, Roth JA. Adenoviral-mediated wild-type p53 gene expression sensitizes colorectal cancer cells to ionizing radiation. *Clin Cancer Res* 1996; **2**: 1665-71.
109. Nielsen LL, Maneval DC. p53 tumor suppressor gene therapy for cancer. *Cancer Gene Ther* 1998; **5**: 52-63.
110. Fisher DE, Bodis S, Lowe S, Takemoto C, Housman D, Jacks T. Restoration of apoptosis in p53 deficient mice. *Blood* 1994; **84** (Suppl 1): abstract 1233.
111. Koechli OR, Schaer GN, Seifert A, Hornung R, Haller U, Eppenberger U, et al. Mutant p53 protein associated with chemosensitivity in breast cancer specimens. *Lancet* 1994; **344**: 1647-8.
112. Garkavtsev I, Grigorian IA, Ossovskaya VS, Chernov MV, Chumakov PM, Gudkov AV. The candidate tumor suppressor p33^{ING1} cooperates with p53 in cell growth control. *Nature* 1998; **391**: 295-8.
113. Oren M. Teaming up to restrain cancer. *Nature* 1998; **391**: 233-4.
114. Kaghad M, Bonnet H, Yang A, Creancier L, Biscan J-C, Valent A, et al. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell* 1997; **90**: 809-19.
115. Jost CA, Marin MC, Kaelin WG Jr. p73 is a human p53-related protein that can induce apoptosis. *Nature* 1997; **389**: 191-4.