BIOCHEMICAL AND MOLECULAR MEDICINE **62,** 3–10 (1997)
ARTICLE NO. MM972616

MINIREVIEW

The Old and the New in p53 Functional Regulation

Lucia Magnelli, Marco Ruggiero, and Vincenzo Chiarugi

Laboratory of Molecular Biology at the Institute of General Pathology, University of Florence, Viale Morgagni 50, 50134 Florence, Italy

Received January 9, 1997

The gene termed p53 is one of the most extensively studied for the past 18 years and the amount of literature published on this gene reflects its relevance in the field of molecular oncology; thus, loss or mutation of this oncosuppressor gene is probably the molecular lesion most frequently observed in human tumors. The aim of this minireview is to report, discuss, and interpret some recent observations on this topic: (I) The relationship with the Ataxia–Telangectasia gene and with the signaling enzyme phosphatidylinositol 3-kinase (PI3K). (II) The relationship between DNA damage, p53, and sensitivity to anticancer therapies. (III) The gain of function caused by mutations that transform the oncosuppressor p53 gene into a dominant transforming oncogene and (IV) The phosphorylative regulation of p53 and its relationship with the mitogenic signaling cascade involving protein kinase C and tumor promoters. © 1997 Academic Press

Key Words: **oncogenes; tumor suppressor genes; signal transduction; ionizing radiations; gene regulation.**

p53 is overexpressed in a variety of spontaneous human tumors and in cultured cells treated with DNA damaging agents. In this second case, experimental evidence indicates that the increase in p53 is regulated at the translational level (i.e., increased synthesis and/or decreased degradation of the protein), rather than at the transcriptional level (for rev. see 1,2); consistently, no changes in mRNA levels are observed (3). Thus, p53 accumulation in the nucleus following DNA damage is blocked by cycloheximide (a protein synthesis inhibitor), but is not affected by actinomycin D (which induces DNA damage by intercalation and inhibits mRNA synthesis) (4).

However, while some functions of p53 associated with immediate responses (such as those involved in the block of the cell cycle and in DNA repair following damage) are regulated at the translational level, other functions of p53 (such as those involved in the regulation of growth and development) require transcriptional regulation. Thus, the p53 gene in the proximity of the first intron shows consensus sequences, termed NF-1 and NF-κB-like, that exhibit promoter activity (5). Regulation of the expression of p53 correlates with both normal cell growth and differentiation: in 3T3 murine fibroblasts cultured *in vitro,* physiological growth stimulation by serum is accompanied by an increase in p53 mRNA (peaking at 23–25 h), followed by increased protein synthesis. *In vivo,* during chicken and murine fetal development the increased expression of p53 during organogenesis is due to increased mRNA synthesis (6,7). Conversely, p53 mRNA decreases in cells induced to differentiate (8–10).

REGULATION OF p53: THE ROLE OF THE ATAXIA–TELANGECTASIA GENE AND OF THE SIGNALING ENZYME PHOSPHATIDYLINOSITOL 3-KINASE (PI3K)

The candidate genes for the transcriptional regulation of p53 are located upstream of the p53 signaling cascade and involve, among others, WAF1 (the

1077-3150/97 $25.00
Copyright © 1997 by Academic Press
All rights of reproduction in any form reserved.

so called *universal cell cycle inhibitor*), GADD45, and cyclin-G. In addition, one of the most interesting genes possibly involved in p53 regulation is the recently cloned ATM gene (Ataxia–Telangectasia Mutated) (11). This gene encodes a chimeric protein consisting of a domain probably functioning as a "sensor" for DNA damage and a carboxy-terminal domain homologous to PI3K. It exhibits significant sequence homology with many yeast genes (MEC-1, RAD3, TEL-1) that are related to the sensitivity to DNA damage induced by radiation and by other means. Indeed, the AT syndrome, characterized by cerebellar degeneration and immunodeficiency, also features increased radiosensitivity, chromosomal instability, and predisposition to cancer development; these latter phenomena are related to deficiences in the induction of p53 by DNA damaging agents. PI3K is a signaling enzyme whose role is still poorly understood: it phosphorylates phosphatidylinositol in position 3 (12), and it also phosphorylates the ribosomal protein pp67s6k (13). PI3K belongs to the cluster of signaling elements which are gathered around the cytoplasmic domains of a variety of activated growth factor receptors with tyrosine kinase activity (12); it is an essential element of the interleukin-2 signaling pathway in lymphoid cells (13). Recently, a possible relationship between p53 and PI3K was also hypothesized (14): it was demonstrated that wortmannin, a specific inhibitor of PI3K, sensitizes murine fibroblasts and human tumor cells to irradiation and blocks the induction of p53 following DNA damage. Thus, wortmannin treatment mimicks some features of the AT syndrome, including radiosensitivity and loss of p53 induction by DNA damage. This might indicate that the PI3K function of the AT gene product is involved in the induction of p53. Although there is no evidence so far that PI3K directly phosphorylates p53, it is known that PI3K also functions as a protein serine kinase; further experiments are required to establish whether the relationship of the AT gene product, PI3K, and p53 also involves other intermediate elements.

Consistent with this hypothesis, it was recently demonstrated that cells from patients with the AT syndrome fail to show the classic G1/S and G2/M delays following irradiation (15). A deficiency in the induction of p53 by ionizing radiation, with consequent lack of WAF1 induction and checkpoint delays, was held responsible for these phenomena. Wild-type p53 was found in AT cells, but some defect in the ATM-dependent cascade impaired its expression; transfection of AT cells with a p53 under the effect of a strong promoter corrected the defect at the G1 checkpoint and restored radiosensitivity.

CHANGES OF p53 HALF-LIFE FOLLOWING DNA DAMAGE: THE RELATIONSHIP BETWEEN DNA DAMAGE, p53, AND SENSITIVITY TO ANTICANCER THERAPIES

p53 accumulates in the nucleus following DNA damage mainly as result of posttranslational modifications increasing the half-life of the protein; however, the nature of the signal responsible for this nuclear shift and accumulation is still uncertain. Newly synthesized p53 accumulates in the cytoplasm during the G1 phase in growth-stimulated 3T3 cells; then the protein localizes in the nucleus at the beginning of the S phase and, just before DNA synthesis begins, it again accumulates in the cytoplasm (16,17); however, when DNA damage occurs, p53 accumulates in the nucleus (18). It appears that the function of p53 as the "guardian of the genome" (1) is somehow "inactivated" by nuclear extrusion during normal DNA synthesis, whereas, following DNA damage, some sort of nuclearization signal induces its accumulation in the nucleus, where it can exert its protective activity eventually leading the cell to either DNA repair or apoptosis (19).

In normal cells p53 shows a relatively short half-life (about 20 min) (20), which can be extended to hours following DNA damage or as a consequence of mutations involving the core domain. PEST sequences (i.e., protein sequences rich in proline, glutamic and aspartic acid, serine, and threonine, involved in the regulation of protein degradation by calcium-dependent proteases), are localized in the core domain between the amino acids in position 211 and 230. As in many other proteins, PEST sequences may be responsible for the short half-life of p53 as they determine its susceptibility to degradation by the ubiquitine pathway (21). DNA damage caused by ionizing radiation at a clinical dose rate causes the rapid accumulation of p53 in the nucleus (22) as a result of new protein synthesis and increased protein stability (3). In addition to ionizing radiation, cisplatin, mytomicin C, and etoposide are among the DNA-damaging agents used in anticancer therapy that induce accumulation of p53 in the nucleus. Nuclear protein accumulation is rapid (be-

ginning a few hours after the treatment) and may remain detectable in surviving cells for more than 10 days. The occurrence of DNA double-strand breaks following treatment appears to be necessary and sufficient to trigger p53 nuclear accumulation (23); consistently, p53 shows intrinsic exonuclease activity (24), which is directly involved in the repair of DNA double-strand breaks, which requires both helicase and exonuclease activities. However, when p53 is mutated, it is unable to perform its role of guardian of the genome as proposed by Lane (1); thus, p53-mutated cells irradiated with ionizing radiation neither repair DNA damage nor undergo apoptosis. The loss of p53 results in resistance to the killing effects of ionizing radiation with a concomitant increase in mutation frequency and genomic instability (25). These phenomena, due to p53 mutations, appear to be critical in neoplastic progression.

THE GAIN OF FUNCTION CAUSED BY CERTAIN MUTATIONS TRANSFORMS THE ONCOSUPPRESSOR GENE p53 INTO A DOMINANT TRANSFORMING ONCOGENE

A number of mutations affect the specific DNA-binding domain located in the core region of p53; among the most frequent are the missense mutations located at amino acids 175, 245, 248, and 273. These mutations do not exert a univocal effect on the functional activity of p53: some of these mutations may affect the turnover rate of p53 and its half-life, and others may cause its loss of function as an oncosuppressor or a dominant negative effect affecting the oncosuppressor activity; alternatively, other mutations may transform p53 into a dominant transforming oncogene, thus causing two tumorigenic steps with a single hit (26).

Mutant forms of p53 show relevant structural changes in the specific DNA-binding core domain which are revealed by specific antibodies (Pab). Wild-type p53 is Pab 240-negative and Pab 1620-positive, while the opposite is true for several p53 mutants (27). These structural changes negatively influence the rapid degradation of p53. Changes in the availability of PEST sequences to proteases might be responsible for impaired degradation (21); however, other mechanisms must also be involved since some MT-p53 (Mutated Transforming p53), which maintain the exposed PEST sequences, show extended half-lives (28).

It is well known that p53 forms oligomers (“dimer of dimers”) and that this quaternary structure strongly favors DNA binding and stabilization (29). The minimal domain required for dimerization was mapped between amino acids 331 and 353 in murine p53 (30); dimerization and tetramerization play a regulative role for both p53 function and specific DNA binding to consensus motifs (31). In addition, two zinc ion binding fingers flank the Pab 240 epitope discovered in MT-p53, and since zinc ions are necessary for normal p53 activity, it is possible that changes in protein conformation affect proper zinc binding with consequent loss of activity.

Thus, most mutations in the p53 gene are single missense point mutations that give rise to altered p53 protein with lost oncosuppressor activity. However, at variance with other classical tumor suppressor genes (i.e., RB or WT1), some mutants of p53 not only lose oncosuppressor activity, but they also exhibit oncogenic transforming functions (26,32). This observation is difficult to reconcile with the dominant-negative model of p53, according to which the coexistence of mutant and wild-type proteins determines the formation of mixed oligomers with impaired oncosuppressor activity because the mutant forms have a dominant-negative effect (33). Thus, transfection of transforming p53 genes into “null” human SAOS-2 or murine BALB-c cells caused increased proliferation and tumorigenicity (34). These data strongly support the hypothesis that certain mutants of p53 not only exhibit loss of function or a dominant-negative effect, but also show a gain of transforming function that confers an active oncogenic potential.

Oncogenic mutant p53 is often nuclearized and it interacts with nuclear substructures such as chromatin and the nuclear matrix, an abundant proteinaceous non-DNA material also involved in the regulation of transcription (35). It was hypothesized that some mutants of p53 transactivate genes involved in growth stimulation and transformation by binding to particular DNA sequences named MAR/SAR (matrix attachment regions/scaffold attachment regions) (36). Thus, other studies showed the interaction of p53 with DNA elements showing MAR/SAR properties (in particular, a 1215-bp *Alu* λ fragment with sequence similarity to MAR/SAR elements). As reported in Fig. 1, MAR elements might operate as a *trait d'union* on the matrix scaffold, joining together elements of transcriptional regulation: the enhancer, the MAR element, the promoter, and the gene that has to be actively transcribed. Muller *et*

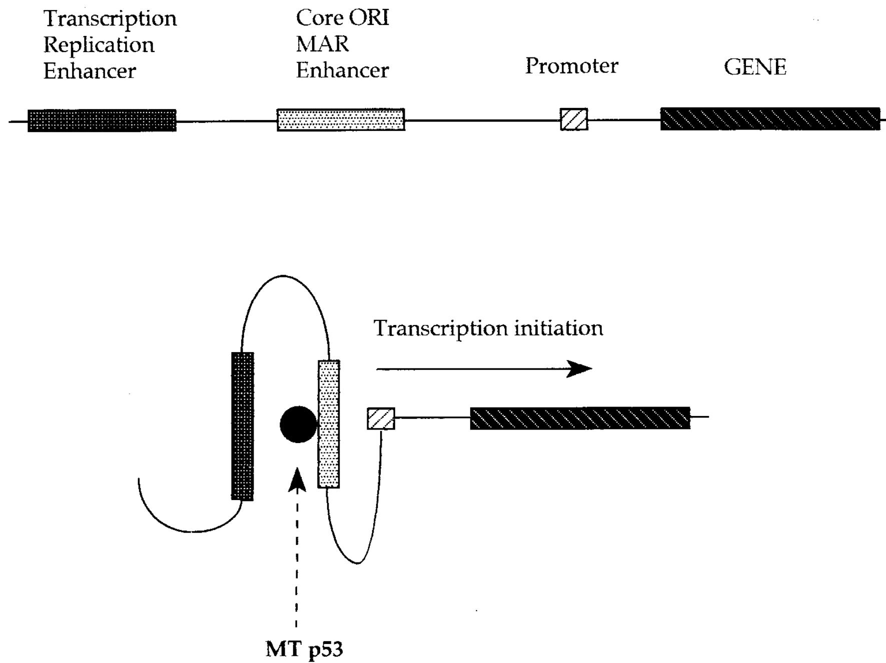

FIG. 1. A model for the functional activity of the AT-rich MAR sequences working as a trait d'union on the matrix scaffold to put in close proximity fundamental elements of transcriptional regulation: enhancers, MARs, promoters, and genes. MT p53 is also considered a MAR-specific activator, as proposed by Muller *et al.* (36).

al. (36) suggest that some mutated forms of p53 with gain of function might influence the transcriptional scaffold complex. Indeed, mutant p53 upregulates certain genes that are involved in cell proliferation and tumorigenesis: interestingly, the same genes are downregulated by wild-type p53. Among the genes upregulated by mutant p53 are IL6; vascular endothelial growth factor (a neoangiogenesis factor involved in the growth of solid tumors); the insulin growth factor I receptor gene (39); PCNA; and the Multi Drug Resistance gene, MDR1, that is responsible for the failure of chemotherapy in a number of tumors (34–38). Thus, the MAR/SAR binding activity of p53 might be among the molecular mechanisms responsible for the gain of function exhibited by some transforming p53 mutants.

In addition to the mutations described above, other mechanisms inactivate the oncosuppressor activity of p53. Thus, blockade of p53 oncosuppressor activity by several DNA viral products is involved in the transforming activity of a large number of DNA viruses. At least six DNA viruses, including papilloma 18/16, SV-4O, adenovirus, Epstein–Barr virus, hepatitis B virus, and cytomegalovirus, encode proteins that impair p53 function with different molecular mechanisms. A possible biological explanation for this phenomenon is that the viral cycle can efficiently proceed only in cells that are deregulated in DNA replication and checkpoints. In addition, a variety of viruses also encode anti-apoptotic genes that protect infected cells from programmed death; this phenomenon involves both the p53-dependent and the p53-independent pathways of apoptosis.

The gene MDM2 was first identified as an amplified gene associated with double minute chromosomes in spontaneously transformed BALB-3T3 murine fibroblasts (40). The human homologue of this gene maps to chromosome 12q13/14 (41), and this chromosomal region is frequently altered in a number of human malignancies. The amplification of MDM2 may represent an alternative mechanism through which transformed cells overcome the p53-regulated growth control. The MDM2 gene product binds to p53 at the amino-terminal domain, thus abrogating the p53-dependent cascade which includes WAF1, GADD-45, Cyclin-G, etc. (42). Momand *et al.* (40) studied the proportion

of p53 bound to MDM2 in cells at different growth levels: in growth-arrested normal cells, 78% of p53 was not bound to MDM2, thus demonstrating that an excess of "free" p53 was necessary for cell cycle arrest; conversely, in actively growing transformed cells, 100% of p53 was bound to MDM2. Phosphorylation of p53 does not appear to influence the binding to MDM2. In conclusion, the binding of MDM2 to p53 in transformed cells suggests this as one of the different mechanisms to override the p53-dependent control.

PHOSPHORYLATIVE REGULATION OF p53 AND ITS RELATIONSHIP WITH THE MITOGENIC SIGNALING CASCADE INVOLVING PROTEIN KINASE C AND TUMOR PROMOTERS

Eight different protein kinases are involved in p53 phosphorylation: casein kinase I and II (43,44), p34cdc2 kinase (45), DNA-activated protein kinase (DNA-PK) (46), MAP kinases (47), protein kinase C (PKC) (30), Raf -1 kinase (48), and Jun-kinase (49).

p34cdc2, an A- and B-cyclin-dependent kinase, phosphorylates human p53 at serine 315: this phosphorylation stimulates the specific binding of DNA to the consensus sequence 5′XXX(AT)(TA)GYYY3′ of p53 (where X is a purine and Y a pyrimidine), and it also causes a distinctive conformational change of the protein, suggesting a potential role in the regulation of checkpoint 2, where p34cdc2 is particularly active. The finding that p34cdc2 phosphorylates p53 is consistent with the recent evidence that p53 is also involved in the regulation of checkpoint 2 (50,51,52).

DNA-PK and MAP kinases appear to be involved in the phosphorylation of human p53 at the amino-terminal domain, influencing the half-life of the protein and its transcriptional activity. DNA-PK appears to be involved also in DNA repair since it contains PI3K motifs just like the AT gene product, which is involved in cell responses to DNA damage. Accordingly, a model for the stabilization of p53 through phosphorylation by DNA-PK in response to DNA damage has been proposed by Selivanova and Winam (53); in this model, p53 binds DNA free ends in association with the DNA-PK Ku subunit that also shows high affinity for DNA free ends. p53 is then phosphorylated by DNA-PK at serine 15–17, thus inducing stabilization and nuclear accumulation. However, recent results (54) suggest that, despite the finding of p53 phosphorylation by DNA-PK *in vitro,* this phenomenon might not play a central role in the activation of p53 as a transcription factor *in vivo.*

The possibility that p53 might exist in a "latent" form was recently proposed (55,56): the model by Hupp *et al.* (55) focuses on the central regulative role played by the carboxy terminal in the tridimensional folding of the p53 tetramer. According to this model, when unphosphorylated, p53 is folded in such a manner that DNA binding to the central functional domain is prevented. The mechanism of phosphorylation at the carboxy terminal seems to be relevant in order to prevent the inactivation of p53, and, interestingly, this phosphorylation is carried out by PKC (55,56,57). The "open" configuration of the four phosphorylated carboxyl ends of the tetramer appears to be a necessary prerequisite for the nonspecific DNA binding that in turn allows the consequent specific DNA binding to p53 consensus motifs (54,55). According to this model the antibody Pab 421 is a useful tool to distinguish between the active and the inactive form of p53: thus, PKC-dependent phosphorylation of serine 378 correlates with the loss of Pab 421 reactivity, which is restored by phosphatases 1 and 2. The phosphorylation of the penultimate amino acid (serine 392) of human p53 is dependent on casein kinase II; however, this site appears to be less critical for p53 activation (58). Furthermore, a very detailed study by Hupp and Lane (59) indicates that phosphorylation of serine 392 can also occur via a casein-kinase II-independent pathway.

According to a recent report (60), phosphorylation of p53 *in vivo* is not performed directly by PKC, but through the involvement of a PKC/MAP kinase pathway. Whatever the case, PKC-dependent, direct or indirect, phosphorylation of serine residues 372–381 at the carboxy terminal of human p53 tetramer is a critical event for the transition from the latent to the active form of p53. Thus, it was recently demonstrated that short treatment with the PKC activator, the tumor promoter PMA (phorbol myristate acetate), causes PKC-dependent phosphorylation of p53 with consequent binding of p53 to DNA (57); however, when PMA treatment is prolonged for more than 12 h (chronic PMA treatment), some isoforms of PKC are downregulated (61,62). In our laboratory we demonstrated that when K562 human leukemia cells, which do not express p53, were transfected with a temperature-sensitive mutant of p53, they underwent apoptosis at the permissive temperature.

In contrast, p53-transfected K562 cells were protected from p53-dependent apoptosis by a chronic PMA treatment, indicating that the apoptotic function of p53 was inactivated by PKC downregulation (63). Therefore, it could be hypothesized that chronic treatment with tumor promoters induces downregulation of PKC with a consequent decrease in p53 phosphorylation and maintenance of p53 in its latent form. Consistent with these observations, cells expressing abnormally low levels of p53 are similar to cells treated with tumor promoters (64); thus, p53-deficient cells as well as cells chronically treated with PMA show instability of the genome at the chromosomal level with possible gene amplification and other gross chromosomal alterations (65–68).

In addition to exogenous tumor promoters such as PMA, endogenous molecules with a possible role as tumor promoters were recently characterized; among these were estrogens, prolactin, and the second messenger diacylglycerol (DAG), which is the physiological activator of PKC. The continuous growth stimulation of cells initiated by carcinogens is likely to be driven by molecules which are produced within the organism itself. DAG is produced by signal-dependent hydrolysis of membrane phospholipids and also as an intermediate product of glycolysis from dihydroxyacetone-phosphate or glycerol-3-phosphate; this metabolic source is quantitatively more relevant, as DAG synthesized *de novo* largely exceeds the amount of DAG produced by receptor-dependent phospholipid hydrolysis (69). We demonstrated that transformation by dominant oncogenes, such as *src* and *ras,* was associated with sustained synthesis *de novo* of DAG (70) with consequent downregulation of PKC (71). Consistently, DAG mimicked the effects of PMA in multi-stage carcinogenesis (72,73), and high levels of DAG were observed in a variety of human tumors (74–76). The hypothesis arising from this evidence is that sustained DAG formation maintains p53 in its latent inactive state: therefore, genomic damage may accumulate and chromosomal aberrations may activate other oncogenes or inactivate the tumor suppressor gene, leading to progression to malignancy.

ACKNOWLEDGMENTS

This study was supported by grants of the Consiglio Nazionale delle Ricerche, Progetto Finalizzato ACRO, and Associazione Italiana per la Ricerca sul Cancro.

REFERENCES

1. Lane DP. The regulation of p53 function. Steiner Award Lecture. *Int J Cancer* **57:**623–627, 1994.
2. Ozbun MA, Butel JS. Tumour p53 mutations and breast cancer: A critical analysis. *Adv Cancer Res* **66:**71–141, 1995.
3. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild type p53 is a cell cycle checkpoint determinant following irradiation. *Proc Natl Acad Sci USA* **89:**7481–7495, 1992.
4. Tishler RB, Calderwood SK, Coleman CM, Price BD. Increases in sequence specific DNA binding by p53 following treatment with chemiotherapeutic and DNA damaging agents. *Cancer Res* **53:**2212–2216, 1993.
5. Ginsberg D, Oren M, Yaniv M, Piette J. Protein binding elements in the promoter region of the mouse p53 gene. *Oncogene* **5:**1285–1290, 1990.
6. Rogel A, Popliker M, Webb CG, Oren M. p53 cellular tumour antigen: Analysis of mRNA levels in normal adult tissues, embryos and tumours. *Mol Cell Biol* **5:**2851–2855, 1985.
7. Louis JM, McFarland VM, May P, Mora PT. The phosphoprotein p53 is down-regulated post-transcriptionally during embryogenesis in vertebrates. *Biochim Biopys Acta* **950:**395–402, 1988.
8. Oren M, Reich NC, Levine AJ. Regulation of the cellular p53 tumour antigen in teratocarcinoma cells and their differentiated progeny. *Mol Cell Biol* **2:**443–449, 1982.
9. Khochbin S, Lawrence J. An antisense RNA involved in p53 mRNA maturation in murine erytroleukemia cells induced to differentiate. *EMBO J* **8:**4107–4114, 1989.
10. Reich NC, Levine A J. Growth regulation of cellular tumour antigen, p53, in non transformed cells. *Nature* **308:**199–201, 1984.
11. Savitzky K, Bar-Shira A, Gilad S, Rotman G, *et al.* A single Ataxia Telangectasia gene with a product similar to PI3K. *Science* **268:**1749–1753, 1995.
12. Carpenter CL, Cantley, LC. Phosphoinositide kinases. *Biochemistry* **29:**11147–11156, 1990.
13. Monfar L, Lemon LC, Grammar, TC, Cheatham L, Vlahos C, Blenis J. Activation of pp70/85 S6 kinase in interleukin 2 responsive lymphoid cells is mediated by phosphatidyl inositol kinase and inhibited by cAMP. *Mol Cell Biol* **15:**326–337, 1995.
14. Price BD, Youmell MB. The phosphatidyl inositol-3-kinase inhibitor wortmannin sensitizes murine fibroblasts and human tumour cells to radiation and blocks induction of p53 following DNA damage. *Cancer Res* **56:**246–250, 1996.
15. Kanna KK, Beamish H, Yan J, Habson K, Williams R, Dunn I, Lavin MF. Nature of G1/S cell cycle checkpoint defect in Ataxia Telangectasia. *Oncogene* **11:**609–618, 1995.
16. Shaulsky G, Goldfinger N, Rotter V. Alteration of tumor development in vivo by the expression of wild type and mutated p53. *Cancer Res* **51:**5232–5237, 1991.
17. Takanashi K, Sukuki K. DNA synthesis associated nuclear exclusion of p53 in normal and human breast epithelial cells in culture. *Oncogene* **9:**183–188, 1994.
18. Frieshe M, Haessler C, Bradner G. Induction of nuclear ac-

cumulation of the tumor suppressor protein p53 by DNA damaging agents. *Oncogene* **8:**307–318, 1993.

19. Lane DP. A death in the life of p53. *Nature* **362:**786–787, 1993.
20. Oren M, Maltzam W, Levine, AJ. Post translational regulation of the 54K cellular tumor antigen in normal and transformed cells. *Mol Cell Biol* **1:**101–110, 1981.
21. Rechsteiner M, Rogers SW. PEST sequences and regulation by proteolysis. *Trends Biochem Sci* **21:**267–271, 1996.
22. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Partecipation of the p53 protein in the cellular response to DNA damage. *Cancer Res* **51:**6304–6311, 1991.
23. Nelson WG, Kastan MB. DNA strand breaks: The DNA template alteration that triggers p53-dependent DNA damage response pathway. *Mol Cell Biol* **14:**1815–1823, 1994.
24. Mumnenbrauer T, James F, Muller B, Wiesmuller L, Depper W, Grosse F. p53 protein exhibits a 3′ to 5′ exonuclease activity. *Cell* **85:**1089–1099, 1996.
25. Lee JM, Bernstein A. p53 mutations increase resistance to ionizing radiations. *Proc Natl Acad Sci USA* **90:**5742–5746, 1993.
26. Deppert W. The yin and yang of p53 in cellular proliferation. *Cancer Biol* **5:**187–202, 1994.
27. Gannon JV, Greaves R, Iggo R, Lane DP. Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for mutant form. *EMBO J* **9:**1595–1602, 1990
28. Stuzbaker HW, Chunakov P, Welch WJ, Jenkins JR. Half-life of mutated p53 isoforms. *Oncogene* **1:**201–211, 1987.
29. Friedman PN, Chen X, Bargonetti J, Prives C. The p53 protein is an unusually shaped tetramer that binds directely to DNA. *Proc Natl Acad Sci USA* **90:**3319–3323, 1993
30. Hupp T, Meek DW, Midgley CA, Lane DP. Regulation of the specific DNA binding function of p53. *Cell* **71:**875–886, 1992.
31. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parkson R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. *Cell* **75:**1–20, 1993.
32. Zambetti GP, Levine AJ. A comparison of the biological activities of wild type and mutant p53. *FASEB J* **7:**855–856, 1993.
33. Milner J, Metcalf EA. Dominant negative mutated p53 isoforms. *Cell* **75:**765–774, 1991.
34. Dittmer D, Pati S, Zambetti G, Chu S, Teresky A, Moore M, Finlay C, Levine AJ. Gain of function mutations in p53. *Nature Genet* **4:**42–46, 1993.
35. Zerrahan J, Deppert W, Weidman D, Patshinsky T, Richards F, Milner J. Correlation between the conformational phenotype of p53 and its subcellular location. *Oncogene* **7:**1371–1381, 1992.
36. Muller BF, Paulsen D, Deppert W. Specific binding of MAR/SAR DNA elements by mutant p53. *Oncogene* **12:**1941–1952, 1996.
37. Margulies L, Shegal PB. Modulation of human interleukin 6 promoter (IL6) and transcription factor C/EBP beta (NF-IL6) activity by p53 species. *J Biol Chem* **268:**15096–15100, 1993.
38. Kieser A, Weich HA, Bradner G, Marm D, Kolch W. Mutant p53 potentiates PKC induction of vascular endothelial growth factor expression. *Oncogene* **9:**963–969, 1994.
39. Werner H, Konieli E, Rauscher F, Le Roth D. Wild type and mutant p53 differently regulate transcription of the insulin like growth factor receptor gene. *Proc Natl Acad Sci USA* **93:**8318–8323, 1996.
40. Momand J, Zambetti GP, Olson D G, George DL, Levine AJ. The MDM2 oncogene products form complexes with the p53 protein and inhibits p53-mediated transactivation. *Cell* **69:**1237–1245, 1992.
41. Oliner JD, Pietempol JA, Tigalingam S, Gyris J, Kinzler, KW, Vogelstein B. Oncoprotein MDM2 cancels the activation domain of tumour suppressor p53. *Nature* **362:**857–860, 1993.
42. Oliner JD, Kinzler KW, Melzer PS, Gerge DL, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* **358:**80–83, 1992.
43. Hupp TR, Lane DP. Regulation of criptic sequence specific DNA binding protein function of p53 by protein kinases. *Cold Spring Harbor Symp Quant Biol* **59:**195–206, 1994.
44. Milne DM, Palmer RH, Meek DW. Mutation of casein kinase II phosphorylation site abolishes the antiproliferative activity of p53. *Nucleic Acids Res* **20:**5560–5570, 1992.
45. Bischoff 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* **87:**4766–4771, 1990.
46. 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* **12:**5041–5046, 1992.
47. Milne DM, Campbell, DG, Caudwell, FD, Meek DW. Phosphorylation of tumor suppressor protein p53 by mitogen activated protein kinases. *J Biol Chem* **269:**9253–9260, 1994.
48. Jamal S, Ziff, EB. Raf phosphorylates p53 in vitro and potentiates p53 transcriptional transactivation in vivo. *Oncogene* **10:**2095–2101, 1995.
49. Milne, DM, Cambell, 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* **270:**5511–5518, 1995.
50. Steward N, Hicks GG, Paraskevas F, Movat M. Evidence for a second cell cycle block at G2/M by p53. *Oncogene* **10:**109–115, 1995.
51. Wang Y, Prives C. Increased and altered DNA binding of human p53 by S and G2/M but not G1 cyclin-dependent kinases. *Nature* **376:**88–91, 1995.
52. Aloni-Grinstein R, Schwartz M, Rotter V.Accumulation of wild type p53 protein upon gamma-irradiation induces a G2 arrest-dependent immunoglobulin K light chain gene expression. *EMBO J* **14:**1392–1401, 1995.
53. Selivanova G, Wiman KG. p53: A cell cycle regulator activated by DNA damage. *Adv Cancer Res* **66:**143–180, 1995.
54. Jongmans W, Artuso M, Vuillaume M, Bresil H, Jackson S P, Hall J. The role of Ataxia Telangectasia and the DNA-dependent protein kinase in p53 mediated cellular response to ionising radiation. *Oncogene* **13:**1133–1138, 1996.

55. Hupp TR, Sparks A, Lane DP. Small peptides activate the latent sequence specific DNA binding function of p53. *Cell* **80:**237–245, 1995.
56. Takenaka I, Morin F, Seizinger BR, and Kley N. Regulation of the sequence-specific DNA binding function of p53 by protein kinase C and protein phosphatases. *J Biol Chem* **270:**5405–5411, 1995.
57. Delphin C, Baudier J. The protein kinase C activator, phorbol ester, cooperates with the wild-type p53 species of ras transformed embryo fibroblasts growth arrest. *J Biol Chem* **269:**29579–29587, 1994.
58. Fishella M, Zambrano N, Ullrich SG, Lin D, Cho B, Mercer E, Anderson CW, Apella E. The carboxyl-terminal serine 392 phosphorylation site of human p53 is not required for wild type activities. *Oncogene* **9:**3249–3252, 1994.
59. Hupp TR, Lane DP. Two distinct signalling pathways activate the latent DNA-binding function of p53 in a casein kinase II-independent manner. *J Biol Chem* **270:**18165–18174, 1995.
60. Milne DM, McKendrick L, Jardine LJ, Deacon E, Lord, JM, Meek DW. Murine p53 is phosphorylated within the Pab 421 epitope by protein kinase C in vitro, but not in vivo, even after stimuli with phorbol ester-*o*-Tetradecanoyl phorbol 13-acetate. *Oncogene* **13:**205–211, 1996.
61. Castagna M. Phorbol esters as signal transducers and tumour promoters. *Biol Cell* **59:**3–13, 1987.
62. Nishizuka Y. The Albert Lasker Medical Awards. The family of protein kinase C for signal transduction. *J Am Med Assoc* **262:**1826–1833, 1989.
63. Magnelli L, Cinelli M, Chiarugi V. Phorbol esters attenuate the expression of p53 in cells treated by doxorubicin and protects ts-p53/K562 from apoptosis. *Biochem Biophys Res Commun* **215:**641–645, 1995.
64. Skouv J, Jensen PO, Forchhammer J, Larsen JK, Lund LR. Tumor -promoting phorbol ester transiently down-modulates the p53 level and blocks the cell cycle. *Cell Growth Differ* **5:**329–340, 1994.
65. Yin Y, Tainsky MA, Bischoff FZ, Strong LC, Wahl M. Wild type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. *Cell* **70:**937–948, 1992.
66. Livingstone LR, White A, Sprouse J, Livanos E, Jaks T, Tlsty TD. Altered cell cycle arrest and gene amplification potential accompany loss of wild type p53. *Cell* **70:**923–935, 1992.
67. Varshavsky A. Phorbol ester dramatically increases incidence of metrotrexate resistant mouse cells: Possible mechanism and relevance to tumor promoters. *Cell* **25:**561–570, 1981.
68. Farber B, Kinzel V. Distribution of phorbol ester TPA induced structural chromosomal aberration in HeLa cells. *Carcinogenesis* **11:**2067–2070, 1990.
69. Farese SV, Konda TS, Davis JR, Standaert ML, Pollet RJ, Cooper DR. Insulin rapidly increases diacylglycerol by activating de novo phosphatidic acid synthesis. *Science* **236:**586–589, 1987.
70. Chiarugi V, Bruni P, Pasquali F, Magnelli L, Basi G, Ruggiero M, Farnararo M. Synthesis of diacylglycerol de novo is responsible for permanent activation and downregulation of protein kinase C in transformed cells *Biochem Biophys Res Commun* **164:**816–823, 1989.
71. Chiarugi V, Magnelli L, Pasquali, Basi G, Ruggiero M. Signal transduction in EJ-H-ras transformed cells: De novo synthesis of diacylglycerol and subversion of agonist stimulated inositol lipid metabolism. *FEBS Lett* **252:**129–134, 1989.
72. Smart RC, Mlls KJ, Hansen LA, Conney AH. Synthetic lipid second messenger sn-1, 2-Didecanoilglycerol: A complete tumor promoter in mouse skin. *Cancer Res* **49:**4455–4458, 1992.
73. Owens MD, Spalding JW, Tennant RW, Smart RC. Genetic alterations cooperate with Ha-ras to accelerate multistage carcinogenesis in TG.AC transgenic mouse skin. *Cancer Res* **55:**3171–3178, 1995.
74. Mills KJ, Reynolds S H, Smart RC. Diacylglycerol is an effector of the clonal expansion of cells containing activated Ha-ras genes. *Carcinogenesis* **14:**2645–2648, 1993.
75. Hendricse C W, Radley S, Donovan DA, Keghley MR, Neoptolemos JP. Activities of phospholipase A2 and diacylglycerol lipase are increased in human colorectal cancer. *Br J Surg* **82:**475–478, 1995.
76. Casamassima F, Pacini S, Dragotto A, Anichini M, Chiarugi V, Ruggiero M. Intracellular diacylglycerol: A mitogenic second messenger proposable as a marker of transformation in squamous cell carcinoma of the lung. *Lung Cancer* **15:**161–170, 1996.