Histol Histopathol (2000) 15: 551-556

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

Histology and Histopathology

Cellular and Molecular Biology

Invited Review

Gain of function properties of mutant p53 proteins at the mitotic spindle cell cycle checkpoint

M.L. Hixon, A. Flores, M. Wagner and A. Gualberto

Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

Summary. Mutations in the p53 tumor suppressor gene locus predispose human cells to chromosomal instability. This is due in part to interference of mutant p53 proteins with the activity of the mitotic spindle and postmitotic cell cycle checkpoints. Recent data demonstrates that wild type p53 is required for postmitotic checkpoint activity, but plays no role at the mitotic spindle checkpoint. Likewise, structural dominant p53 mutants demonstrate gain-of-function properties at the mitotic spindle checkpoint and dominant negative properties at the postmitotic checkpoint. At mitosis, mutant p53 proteins interfere with the control of the metaphase-to-anaphase progression by up-regulating the expression of CKsl, a protein that mediates activatory phosphorylation of the anaphase promoting complex (APC) by Cdc2. Cells that carry mutant p53 proteins overexpress CKsl and are unable to sustain APC inactivation and mitotic arrest. Thus, mutant p53 gain-of-function at mitosis constitutes a key component to the origin of chromosomal instability in mutant p53 cells.

Key words: p53, Cyclin B, CKsl, CKsHs1, Cell cycle checkpoint

Introduction

The genesis of cancer is a multistep process that requires a normal cell to undergo a series of changes in order to progress to a tumorigenic state (Bishop, 1987). It has been postulated that both the generation of new cellular variants and their progression to neoplasia are originated by the acquisition of genomic instability (Nowell, 1976). Genomic instability is frequently initiated in preneoplastic cells by loss of fidelity in the processes that replicate, repair, and segregate the genome (Hartwell and Kastan, 1994), allowing for the accumulation of genetic alterations that lead to a malignant phenotype. Therefore, it is becoming increasingly evident that loss of the mechanisms that maintain the integrity of the human genome is a primary event in the genesis of cancer.

Research addressing the relationship between cell proliferation and the maintenance of genomic integrity in human cells is relatively recent. Extensive evidence demonstrates that the onset of every phase of the cell cycle depends critically on the adequate completion of the previous cell cycle phase. The coordination of these events is carried out by pathways called cell cycle checkpoints (Elledge, 1996). These pathways oversee the progression of the cell cycle and play a crucial role in the maintenance of genomic integrity by coordinating the cell cycle regulatory machinery with DNA repair and cell death pathways (Tlsty et al., 1995). The first set of checkpoints occurs at the G1/S transition and results in a delay in the progression from G1 into S phase preventing the replication of damaged DNA (Weinert, 1998). A second set of checkpoints associated with DNA repair activities may occur during S phase. The dependence of mitosis on the completion of the period of DNA replication in the cell cycle ensures that chromosome segregation takes place only after the genome has been fully duplicated (Agarwal et al., 1998; Michael and Newport, 1998). A third set of checkpoints is located at M phase and the G2/M transition and results in a delay in the progression into and out of mitosis (Minshull et al., 1994; Murray, 1995). In this review, we will discuss the role that mutations of the p53 tumor suppressor gene product, a key regulator of cell growth (reviewed in Lopez-Saez et al., 1998), plays in the control of the progression through mitosis.

Discussion

At mitosis, the proper segregation of chromosomes requires the execution of a number of processes: a bipolar spindle must be assembled, chromosomes must be attached to the spindle through the kinetochore, and properly attached chromosomes must align at the metaphase plate. The mitotic spindle cell cycle checkpoint prevents the onset of anaphase, the actual

Offprint requests to: Dr. A. Gualberto, Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, 10900 Euclid Ave., Cleveland, Ohio 44106. USA. Fax: (216) 368 3952. e-mail: axg29@po.cwru.edu

segregation of chromosomes, until all these events have been properly completed (DeWald et al., 1994). In addition, cells with anomalous mitosis that escape the control of the mitotic checkpoint may be growth arrested (Stewart et al., 1999) or destroyed (Lanni and Jacks, 1998) at a tetraploid G1 phase by the postmitotic checkpoint, a DNA damage-responsive pathway (Fig. 1). Loss of mitotic spindle cell cycle checkpoint status results in cell death and/or aneuploidy, a hallmark of tumor progression (Cahill et al., 1998).

Recent reports have suggested a role for p53 at the mitotic spindle cell cycle checkpoint. Fibroblasts isolated from Li-Fraumeni syndrome (LFS) individuals, who are born with heterozygous mutations in the p53 tumor suppressor gene (Malkin et al., 1990; Srivastava et al., 1990), have a marked tendency to become heteroploid in culture (Bischoff et al., 1990). Heteroploidy is also commonly found in p53 null mouse cells in culture (Harper et al., 1993), and in p53-knockout mice (Cross et al., 1995). Also, the expression of mutant p53 proteins in human colon carcinoma cells and murine cell lines causes karyotypic abnormalities, including an increase in ploidy levels during growth in culture (Agapova et al., 1996). Intriguingly, overexpression of a mutant p53 protein on a p53 null background accelerated the appearance of polyploidy in a myelomonocytic cell line (Peled et al., 1996). Moreover, we have seen that LFS fibroblasts that carry structural dominant p53 mutant proteins progress quickly to polyploidy when incubated in the presence of mitotic spindle inhibitors (Gualberto et al., 1998). However, normal human fibroblasts, p53 null LFS fibroblasts, or normal human fibroblasts carrying the human papilloma virus 16 E6 (HPV16 E6), that binds and promotes the degradation of p53, are able to arrest growth in response to mitotic inhibitors (Gualberto et al., 1998). Also, Jacks and coworkers reported that p53 null mouse fibroblasts have a normal mitotic spindle checkpoint (Lanni and Jacks, 1998). Therefore, according to these results, the abrogation of the mitotic spindle cell cycle checkpoint induced by structural mutant p53 proteins is a gain-of-function property that can not be explained by the loss of wild type p53 function. However, polyploidy has been reported by others in HPV16 E6-expressing human fibroblasts and in p53 null mouse fibroblasts (Cross et al., 1995; Di Leonardo et al., 1997). The reason for these apparently contradictory results may be the existence of profound differences in the mechanisms that underlie the onset of aneuploidy in p53 null and mutant p53-expressing cells. We and others have shown that the mitotic spindle cell cycle checkpoint is transient in p53 null cells (Hixon et al., 1998; Lanni and Jacks, 1998). Thus, these cells may eventually exit mitotic arrest and become polyploidy due to inactivation of a p53-dependent postmitotic checkpoint (Minn et al., 1996; Di Leonardo et al., 1997; Lanni and Jacks, 1998) (Fig. 2). In conclusion, loss of p53 protein may result in aneuploidy due to lack of postmitotic checkpoint activity while structural dominant p53 mutants may both abrogate the mitotic checkpoint (gain-of-function) and work as dominant negative mutants at the postmitotic checkpoint.

How can p53 mutant proteins affect the control of mitosis? The progression through mitosis is regulated by the activity of a complex referred as the Maturation or M-phase Promoting Factor (MPF). This complex

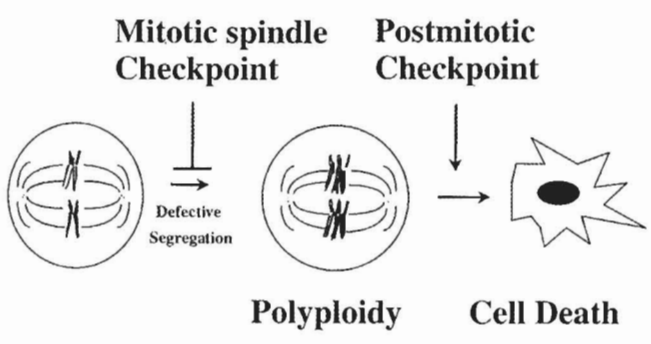

Fig. 1. Cells are protected from polyploidy by the activity of the mitotic spindle and postmitotic cell cycle checkpoints.

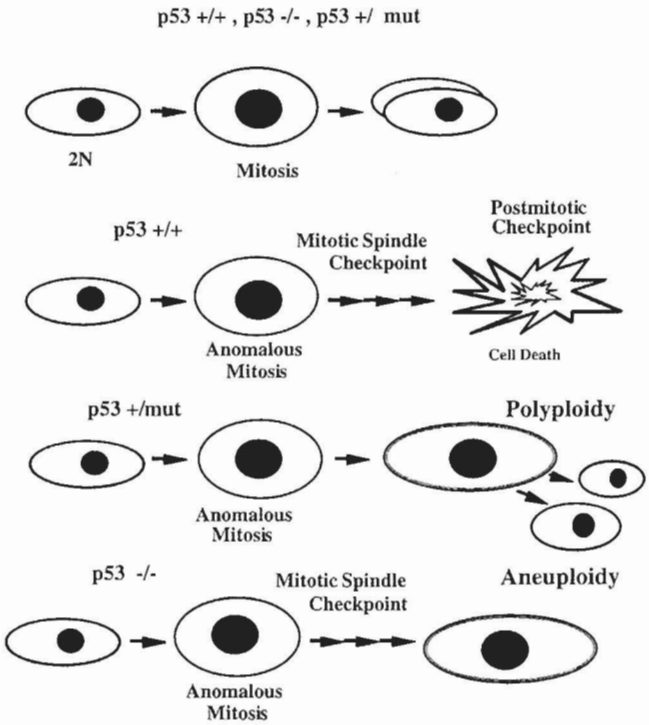

Fig. 2. In response to an anomalous chromosomal segregation, normal cells (p53 +/+) delay transiently the exit from mitosis (mitotic spindle checkpoint). p53 +/+ cells with non-segregated chromosomes may eventually re-enter the cell cycle but are destroyed at the subsequent G1 phase (postmitotic checkpoint, an apoptotic pathway). Loss of p53 (p53 -/-) abrogates the postmitotic cell cycle checkpoint whereas gain of a dominant p53 mutant (p53 +/mut) abrogates both the mitotic and postmitotic checkpoints. Loss of p53 function, either by loss of p53 (p53 -/-) or mutant p53 dominance (p53 +/mut), may then originate aneuploidy due to centrosome amplification (Fukasawa et al., 1996) and multipolar division.

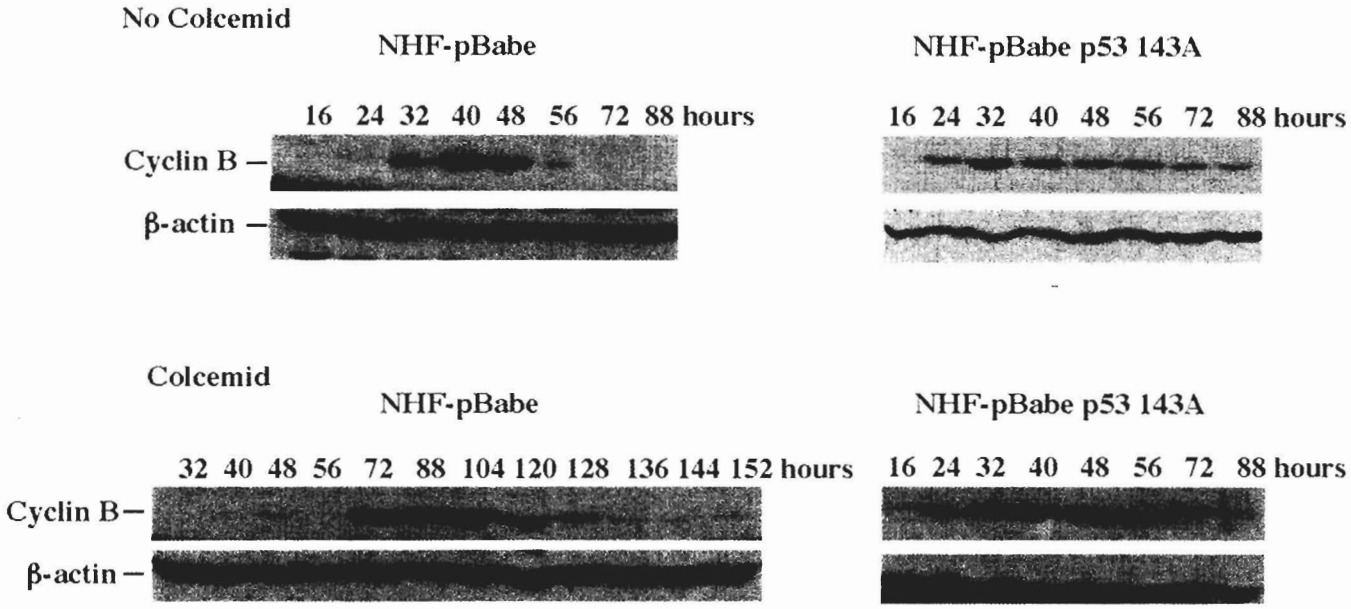

Fig. 3. The expression of a mutant p53 protein in normal human fibroblasts abrogates the ability of these cells to delay Cyclin B degradation in response to mitotic spindle depolymerization. Normal human fibroblasts infected with an empty retrovirus (pBabe) incubated in media with colcemid, a mitotic spindle depolymerizing agent, delay Cyclin B degradation up to 80 hours. This delay in Cyclin B metabolism represents the activity of the mitotic spindle checkpoint and is lost when human fibroblasts are infected with a retrovirus that drives the expression of a dominant p53 mutant (pBabe p53 143A) (Hixon et al., 1998).

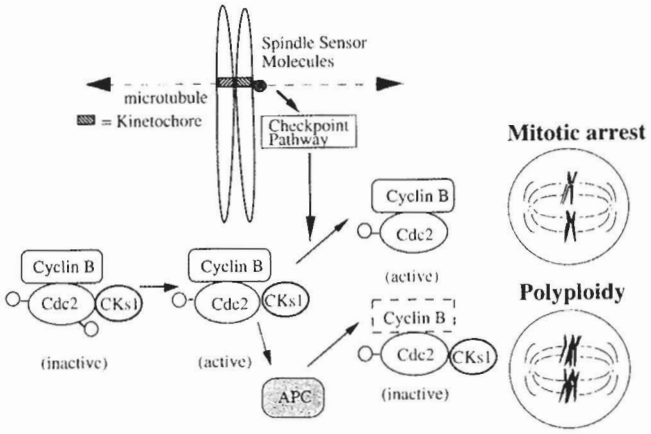

Fig. 4. Model of the mitotic spindle cell cycle checkpoint pathway. Cdc2 phosphorylates and activates the protease complex APC. CKs1 allows docking of Cdc27 onto Cdc2. Dowregulation of CKs1 expression by the mitotic spindle checkpoint pathway blocks APC activation and degradation of its mitotic targets such as Cyclin B.

consists of a catalytic subunit (34 kD cyclin dependent-kinase, Cdc2), a regulatory subunit (Cyclin B proteins), and associated proteins (Norbury and Nurse, 1992). Entry into mitosis requires MPF activation that depends on Cyclin B expression and activatory dephosphorylation of Cdc2. Progression through mitosis and cytokinesis (cell division) requires the inactivation of MPF that is accomplished in part by Cyclin B degradation. Recent data demonstrate that the mutant p53 proteins render human cells unable to delay Cyclin B metabolism in response to mitotic spindle depolymerization (Fig. 3). Moreover, this effect of mutant p53 proteins cannot be reproduced by the inactivation of wild type p53 (Hixon et al., 1998). How do mutant p53 proteins affect the metabolism of Cyclin B and other mitotic factors? Little is known about the molecular events that constitute the mitotic spindle cell cycle checkpoint pathway. However, biochemical and genetic evidence demonstrates that mitotic spindle checkpoint signals, originated in part by the association of MAD2 proteins to unattached kinetochores (Chen et al., 1996; Li and Benezra, 1996; Pangilinan and Spencer, 1996), are transduced to modulate Cyclin B metabolism and MPF activity (Murray et al., 1996). Thus, Cyclin B is degraded and MPF is inactivated only after certain aspects of mitosis related to spindle assembly and disassembly are properly completed (Basi and Draetta, 1995a,b). Recent data indicates that MAD2 associates with the Cdc27 component of the anaphase promoting complex (APC), a protease that targets Cyclin B and other mitotic targets, inhibiting its proteolytic activity (Fang et al., 1998; Hwang et al., 1998; Wassmann and Benezra, 1998). On the other hand, Cdc27-APC is activated by MPF in a reaction that requires its association with the Cdc2-CKs1 complex (Patra and Dunphy, 1998) (Fig. 4). Extensive evidence indicates that the level of CKs1 or homologous proteins is rate limiting in Cyclin B degradation (Moreno et al., 1989; Basi and Draetta, 1995b; Patra and Dunphy, 1996, 1998;

Hixon et al., 1998). CKs1, also called CKsHs1 for Cdc2/Cdc28 kinase subunit *Homo sapiens* 1 (Richardson et al., 1990), was initially identified as a human homologue of the Cdc28/Cdc2-associated proteins of *S. cerevisiae*, CKs1, and *S. pombe*, Suc1 (Richardson et al., 1990). Importantly, in *S. pombe*, inactivation of the suc 1 gene causes mitotic arrest with high levels of cdc13 (Cyclin B homologue) and high MPF kinase activity (Moreno et al., 1989; Basi and Draetta, 1995b). We have observed that downregulation of the expression of CKs1 is required for mitotic arrest in human fibroblasts, that cells carrying p53 mutants overexpress and fail to downregulate CKs1 in response to mitotic checkpoint signals, and that the ectopic expression of CKs1 in normal cells promotes unscheduled degradation of Cyclin B and polyploidy (Hixon et al., 1998). These data identify CKs1 as a mutant p53 gene target and demonstrate that the unregulated expression of CKs1 in mutant p53 cells leads to the abrogation of the mitotic spindle cell cycle checkpoint. Importantly, CKs1 is the first identified mutant p53 target at the mitotic spindle checkpoint. A wild type p53-regulated gene, p21cip, has also been implicated in the mitotic spindle cell cycle checkpoint. However, p21 expression is controlled by both p53-dependent and p53-independent mechanisms, and recent data indicate that the p21 G2/M growth arrest does not correlate with p53 status (Niculescu et al., 1998). The p21 gene may be implicated in a postmitotic growth arrest (Stewart et al., 1999). Transcription of the G2/M cell cycle regulator 14-3-3 has been shown to be regulated by p53 (Hermeking et al., 1998). In addition, wild type p53 negatively regulates the expression of Map4 (Murphy et al., 1996), a microtubule-associated protein that interacts with Cyclin B (Ookata et al., 1993; Murphy et al., 1996).

How can mutant p53 proteins affect the expression of CKs1 and other mitotic factors? In principle, mutant p53 could activate directly the expression of CKs1. In that respect, gain-of-function transcriptional properties have been described already for some p53 mutants (Subler et al., 1992; Dittmer et al., 1993; Gualberto et al., 1995; Lin et al., 1995; Frazier et al., 1998). Mutant p53 may also regulate CKs1 expression indirectly, affecting the levels of a CKs1 regulatory factor(s). Recent data from Prives and coworkers (Di Como et al., 1999) and from our laboratory (Ruiz-Lozano et al., 1999) indicate a molecular mechanism that could explain the gain-of-function properties of mutant p53 proteins at mitosis. We have seen that dominant mutant forms of p53 are able to block the ability of the p53-related proteins p51 and p73 to transactivate wild type p53 gene targets. These results indicate that the dominant negative activity of mutant p53 can be exerted not only over wild type p53, but also over other p53-related proteins. These data suggest also that certain differences between the mutant p53 and the p53 null phenotypes could be originated by the ability of p53-related proteins to compensate for the lack of p53 function in the latter.

Conclusions

The identification of CKs1 as a mutant p53 gene target provides a key component to our understanding of the origin of heteroploidy in mutant p53 expressing cells. The fact that CKs1 and related proteins may play a key role at the mitotic spindle cell cycle checkpoint was postulated previously (Rudner and Murray, 1996), but direct evidence has been lacking until now. In spite of recent data, many details remain to be elucidated. Whether mutant p53 directly regulates CKs1 or affects the control of its expression through interaction with p53-related proteins warrants further investigation. Future work should be also directed to determine the role of the upregulation of CKs1 and other members of this family of proteins in human tumor initiation and progression.

Acknowledgments. This work was supported in part by grants from the Ohio Cancer Research Associates and the American Heart Association to A.G.

References

Agapova L.S., Ilyinskaya G.V., Turovets N.A., Ivanov A.V., Chumakov P.M. and Kopnin B.P. (1996). Chromosome changes caused by alterations of p53 expression. Mutat. Res. 354, 129-138.

Agarwal M.L., Agarwal A., Taylor W.R., Chernova O., Sharma Y. and Stark G.R. (1998). A p53-dependent S-phase checkpoint helps to protect cells from DNA damage in response to starvation for pyrimidine nucleotides. Proc. Natl. Acad. Sci. USA 95, 14775-14780.

Basi G. and Draetta G. (1995a). The Cdc2 kinase:structure, activation, and its role at mitosis in vertebrate cells. In: Cell cycle control. Hutchison G. and Glover D.M. (eds). Oxford University Press, Oxford. pp 106-134.

Basi G. and Draetta G. (1995b). p13suc1 of *Schizosaccharomyces pombe* regulates two distinct forms of the mitotic Cdc2 kinase. Mol. Cell. Biol. 15, 2028-2036.

Bischoff F.Z., Yim S.O., Pathak S., Grant G., Siciliano M.J., Giovanella B.C., Strong L.C. and Tainsky M.A. (1990). Spontaneous abnormalities in normal fibroblasts from patients with Li-Fraumeni cancer syndrome: aneuploidy and immortalization. Cancer Res. 50, 7979-7984.

Bishop J.M. (1987). The molecular genetics of cancer. Science 235, 305-311.

Cahill D.P., Lengauer C., Yu J., Riggins G.J., Wilson J.K., Markowitz S.D., Kinzler K.W. and Vogelstein B. (1998). Mutations of mitotic checkpoint genes in human cancers. Nature 392, 300-303.

Chen R.H., Waters J.C., Salmon E.D. and Murray A.W. (1996). Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores. Science 274, 242-246.

Cross S.M., Sanchez C.A., Morgan C.A., Schimke M.K., Ramel S., Idzerda R.L., Raskind W.H. and Reid B.J. (1995). A p53-dependent mouse spindle checkpoint. Science 267, 1353-1356.

DeWald M.G., Sharma R.C., Kung A.L., Wong H.E., Sherwood S.W. and Schimke R.T. (1994). Heterogeneity in the mitotic checkpoint control of BALB/3T3 cells and a correlation with gene amplification propensity. Cancer Res. 54, 5064-5070.

Di Leonardo A., Khan S.H., Linke S.P., Greco V., Seidita G. and Wahl G.M. (1997). DNA rereplication in the presence of mitotic spindle inhibitors in human and mouse fibroblasts lacking either p53 or pRb function. Cancer Res. 57, 1013-1019.

Dittmer D., Pati S., Zambetti G., Chu S., Teresky A.K., Moore M., Finlay C. and Levine A.J. (1993). Gain of function mutations in p53. Nat. Genet. 4, 42-46.

Elledge S.J. (1996). Cell cycle checkpoints: preventing an identity crisis. Science 274, 1664-1672.

Fang G., Yu H. and Kirschner M.W. (1998). The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation. Genes Dev. 12, 1871-1883.

Frazier M.W., He X., Wang J., Gu Z., Cleveland J.L. and Zambetti G.P. (1998). Activation of c-myc gene expression by tumor-derived p53 mutants requires a discrete C-terminal domain. Mol. Cell. Biol. 18, 3735-3743.

Fukasawa K., Choi T., Kuriyama R., Rulong S. and Vande Woude G.F. (1996). Abnormal centrosome amplification in the absence of p53. Science 271, 1744-1747.

Gualberto A., Aldape K., Kozakiewicz K. and Tlsty T.D. (1998). An oncogenic form of p53 confers a dominant, gain-of-function phenotype that disrupts spindle checkpoint control. Proc. Natl. Acad. Sci. USA 95, 5166-5171.

Gualberto A., Hixon M.L., Finco T.S., Perkins N.D., Nabel G.J. and Baldwin A.S. Jr. (1995). A proliferative p53-responsive element mediates tumor necrosis factor alpha induction of the human immunodeficiency virus type 1 long terminal repeat. Mol. Cell. Biol. 15, 3450-3459.

Harper J.W., Adami G.R., Wei N., Keyomarsi K. and Elledge S.J. (1993). The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75, 805-816.

Hartwell L.H. and Kastan M.B. (1994). Cell cycle control and cancer. Science 266, 1821-1828.

Hermeking H., Lengauer C., Polyak K., He T.-C., Zhang L., Thiagalingam S., Kinzler K.W. and Vogelstein B. (1998). 14-3-3σ is a p53-regulated inhibitor of G2/M progression. Mol. Cell 1, 3-11.

Hixon M.L., Flores A.I., Wagner M.W. and Gualberto A. (1998). Ectopic expression of Cdc2/Cdc28 kinase subunit homo sapiens 1 uncouples Cyclin B metabolism from the mitotic spindle cell cycle checkpoint. Mol. Cell. Biol. 18, 6224-6237.

Hwang L.H., Lau L.F., Smith D.L., Mistrot C.A., Hardwick K.G., Hwang E.S., Amon A. and Murray A.W. (1998). Budding yeast Cdc20: a target of the spindle checkpoint. Science 279, 1041-1044.

Lanni J.S. and Jacks T. (1998). Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. Mol. Cell. Biol. 18, 1055-1064.

Li Y. and Benezra R. (1996). Identification of a human mitotic checkpoint gene: hsMAD2. Science 274, 246-248.

Lin J., Teresky A.K. and Levine A.J. (1995). Two critical hydrophobic amino acids in the N-terminal domain of the p53 protein are required for the gain of function phenotypes of human p53 mutants. Oncogene 10, 2387-2390.

Lopez-Saez J.F., De la Torre J., Pincheira J. and Gimenez-Martin G. (1998). Cell proliferation and cancer. Histol. Histopathol. 13, 1197-1214.

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. and Tainsky M.A. (1990). Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250, 1233-1238.

Michael W.M. and Newport J. (1998). Coupling of mitosis to the completion of S phase through Cdc34-mediated degradation of Wee1. Science 282, 1886-1889.

Minn A.J., Boise L.H. and Thompson C.B. (1996). Expression of Bcl-xL and loss of p53 can cooperate to overcome a cell cycle checkpoint induced by mitotic spindle damage. Genes Dev. 10, 2621-2631.

Minshull J., Sun H., Tonks N.K. and Murray A.W. (1994). A MAP kinase-dependent spindle assembly checkpoint in *Xenopus* egg extracts. Cell 79, 475-486.

Moreno S., Hayles J. and Nurse P. (1989). Regulation of p34Cdc2 protein kinase during mitosis. Cell 58, 361-372.

Murphy M., Hinman A. and Levine A.J. (1996). Wild-type p53 negatively regulates the expression of a microtubule-associated protein. Genes Dev. 10, 2971-2980.

Murray A.W. (1995). The genetics of cell cycle checkpoints. Curr. Opin. Genet. Dev. 5, 5-11.

Murray A.W., Desai A.B. and Salmon E.D. (1996). Real time observation of anaphase in vitro. Proc. Natl. Acad. Sci. USA 93, 12327-12332.

Niculescu A.B., Chen X., Smeets M., Hengst L., Prives C. and Reed S.I. (1998). Effects of p21 (cip/Waf1) at both the G1/S and the G2/M cell cycle transitions: pRb is a critical determinent in blocking DNA replication and in preventing endoreduplication. Mol. Cell. Biol. 18, 629-643.

Norbury C. and Nurse P. (1992). Animal cell cycles and their control. Annu. Rev. Biochem. 61, 441-470.

Nowell P.C. (1976). The clonal evolution of tumor cell populations. Science 194, 23-28.

Ookata K., Hisanaga S., Okumura E. and Kishimoto T. (1993). Association of p34Cdc2/Cyclin B complex with microtubules in starfish oocytes. J. Cell. Sci. 105, 873-881.

Pangilinan F. and Spencer F. (1996). Abnormal kinetochore structure activates the spindle assembly checkpoint in budding yeast. Mol. Biol. Cell. 7, 1195-1208.

Patra D. and Dunphy W.G. (1996). Xe-p9, a Xenopus Suc1/Cks homolog, has multiple essential roles in cell cycle control. Genes Dev. 10, 1503-1515.

Patra D. and Dunphy W.G. (1998). Xe-p9, a Xenopus Suc1/Cks protein, is essential for the Cdc2-dependent phosphorylation of the anaphase-promoting complex at mitosis. Genes Dev. 12, 2549-2559.

Peled A., Schwartz D., Elkind N.B., Wolkowicz R., Li R. and Rotter V. (1996). The role of p53 in the induction of polyploidity of myelo-monocytic leukemic M1/2 cells. Oncogene 13, 1677-1685.

Richardson H.E., Stueland C.S., Thomas J., Russell P. and Reed S.I. (1990). Human cDNAs encoding homologs of the small p34Cdc28/Cdc2-associated protein of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Genes Dev. 4, 1332-1344.

Rudner A.D. and Murray A.W. (1996). The spindle assembly checkpoint. Curr. Opin. Cell. Biol. 8, 773-780.

Ruiz-Lozano P., Hixon M.L., Wagner M.W., Flores A., Chien K., Baldwin A.S. and Gualberto A. (1999). p53 is a transcriptional activator of the muscle-specific phosphoglycerate mutase gene and contributes in vivo to the control of its cardiac expression. Cell. Growth Diff. (in press).

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

Stewart Z.A., Leach S.D. and Pietenpol J.A. (1999). p21(Waf1/Cip1) inhibition of cyclin E/Cdk2 activity prevents endoreduplication after mitotic spindle disruption. Mol. Cell. Biol. 19, 205-215.

Subler M.A., Martin D.W. and Deb S. (1992). Inhibition of viral and cellular promoters by human wild-type p53. J. Virol. 66, 4757-4762.

Tlsty T.D., Briot A., Gualberto A., Hall I., Hess S., Hixon M., Kuppuswamy D., Romanov S., Sage M. and White A. (1995). Genomic instability and cancer. Mutat. Res. 337, 1-7.

Wassmann K. and Benezra R. (1998). Mad2 transiently associates with an APC/p55Cdc complex during mitosis. Proc. Natl. Acad. Sci. USA 95, 11193-11198.

Weinert T. (1998). DNA damage checkpoints update: getting molecular. Curr. Opin. Genet. Dev. 8, 185-193.

Accepted September 21, 1999