*Review*

# The Role of the Cyclin-dependent Kinase Inhibitor p21 in Apoptosis[1]

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## Abstract

**Cancer develops when the balance between cell proliferation and cell death is disrupted, and the ensuing aberrant proliferation leads to tumor growth. The cyclin-dependent kinase inhibitor p21 is induced by both p53-dependent and -independent mechanisms following stress, and induction of p21 may cause cell cycle arrest. As a proliferation inhibitor, p21 is poised to play an important role in preventing tumor development. This notion is supported by data indicating that p21-null mice are more prone to spontaneous and induced tumorigenesis, and p21 synergizes with other tumor suppressors to protect against tumor progression in mice. However, a number of recent studies have pointed out that in addition to being an inhibitor of cell proliferation, p21 acts as an inhibitor of apoptosis in a number of systems, and this may counteract its tumor-suppressive functions as a growth inhibitor. In the current review, we discuss the role of p21 in regulating cell death and the potential relevance of its expression in cancer.**

## Introduction

Sequential activation of cyclin/Cdk[3] complexes regulates progression through the cell cycle (reviewed in Refs. 1–3). Active cyclin/Cdk complexes phosphorylate and inactivate members of the retinoblastoma protein (Rb) family that are negative regulators of $G_1$ and S-phase progression, leading to induction of E2F-regulated gene expression and cell proliferation. CKIs bind and inhibit the activity of cyclin/Cdk complexes and negatively regulate cell cycle progression (reviewed in Refs. 4, 5). These proteins play important roles regulating proliferation during normal development and differentiation and after genotoxic stress.

p21 (also called WAF1, CAP20, Cip1, and Sdi1; Refs. 6–9) is the founding member of the Cip/Kip family of CKIs, which also includes p27 (10, 11) and p57 (12, 13). These three CKIs contain a conserved region of sequence at the $NH_2$ terminus that is required and sufficient for the inhibition of cyclin/Cdk complexes, whereas the COOH terminal regions are variable in length and function (12, 14–16). They can bind and inhibit a broad range of cyclin/Cdk complexes, with a preference for those containing Cdk2 (17, 18). p21 plays an essential role in growth arrest after DNA damage (19–21), and overexpression leads to $G_1$ and $G_2$ (22) or S-phase (23) arrest.

Although one molecule of p21 is sufficient to inhibit cyclin/Cdk complexes (22), Cip/Kip CKIs have been detected in active cyclin D/Cdk4 complexes (24–27). p21 was shown to stabilize interactions between Cdk4 and cyclin D and promote the formation of active complexes in a concentration-dependent manner (27). In glioma (28) and in vascular smooth muscle cells (29), p21 facilitates active cyclin-Cdk complex formation and induces cell proliferation. Support for Cip/Kip proteins in promoting Cdk4 activity *in vivo* came from studies with p21/p27-deficient MEFs because these cells contained undetectable levels of cyclin D/Cdk4 complexes (30). However, the p21/p27-deficient cells retained some sensitivity to the INK4 CKI p16, which specifically inhibits cyclin D/Cdk4 complexes, suggesting the presence of some active cyclin D/Cdk4 complexes (30). In addition, active cyclin D3/Cdk4 complexes were detected in p21/p27-deficient MEFs in another study (31), also indicating that other factors may influence assembly and activation of cyclin D/Cdk4 complexes *in vivo*.

Several studies suggest that Cip/Kip CKIs may be sequestered in cyclin D-containing complexes. In cells engineered to express mitogen-activated protein kinase (MEK1) and cyclin D/Cdk, p27 was recruited into cyclin D1/Cdk4 complexes, and Cdk2-containing complexes were activated (32). In other studies, Myc was found to activate cyclin E/Cdk2, in part by inducing expression of cyclin D1 and cyclin D2, which associate with and sequester p27 and p21 in proliferating cells (33, 34). Ectopic expression of the INK4 CKI p16 inhibited Myc-induced dissociation of p27 from cyclin E/Cdk2 (34). Cdk4, Cdk6, and Cdk2 activity was inhibited in U2-OS cells engineered to overexpress p16 (35). In these cells, p27 was redistributed from Cdk4- to Cdk2-containing complexes upon induction of p16. Thus, in addition to inhibiting Cdk4 through direct binding, INK4 CKIs may indirectly regulate Cdk2 activity by displacing sequestered Cip/Kip proteins from cyclin D-containing complexes. Genetic evidence for cyclin D/Cdk4 interactions with Cip/Kip inhibitors has also been obtained from studies in knockout mice (36–38).

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[3] The abbreviations used are: Cdk, cyclin-dependent kinase; Rb, retinoblastoma; CKI, cyclin kinase inhibitor; MEF, mouse embryonic fibroblast; PCNA, proliferating cell nuclear antigen; TGF, transforming growth factor; TNF, tumor necrosis factor; NF-κB, nuclear factor-κB; SAHA, suberoylanilide hydroxamic acid; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; TRAIL, TNF-related apoptosis-inducing ligand; PDTC, pyrrolidine dithiocarbamate; 5-FU, 5-fluorouracil.

In addition to binding cyclin/Cdk complexes, p21 contains a COOH terminal binding site for PCNA and is found in quaternary complexes containing p21, PCNA, and cyclin/Cdk in normal cells (8, 18, 39). PCNA plays an essential role in DNA replication and different types of DNA repair, including nucleotide excision repair, mismatch repair, and base excision repair (reviewed in Refs. 40, 41). Through its direct interaction with PCNA, p21 can block DNA synthesis by DNA polymerase $\delta$ (42–44). The functions of p21 in regulating DNA repair through its interactions with PCNA and associated proteins remain somewhat controversial. Different approaches have led investigators to conclude that p21 is required (45, 46), unnecessary (47–49), and inhibitory (50, 51) for DNA repair, with nucleotide excision repair as the general focus of these studies. Mismatch repair activity was shown to be inhibited by p21 or a p21 peptide that bound PCNA, and this inhibition could be reversed by increasing levels of PCNA in the reaction (52). Recently, p21 was found to inhibit PCNA-stimulated long patch base excision repair (53).

p21 expression has been shown to be regulated largely at the transcriptional level by both p53-dependent and -independent mechanisms (reviewed in Ref. 54). The p21 promoter contains two conserved p53-binding sites, and at least one of these is required for p53 responsiveness after DNA damage (55). In addition, a variety of transcription factors that are induced by a number of different signaling pathways activate p21 transcription by p53-independent mechanisms, including Sp1, Sp3, Ap2, STATs, C/EBP$\alpha$, C/EBP$\beta$, and the bHLH proteins BETA2 and MyoD (reviewed in Ref. 54). p21 expression may also be regulated posttranscriptionally by both ubiquitin-dependent and -independent proteasome-mediated degradation (56–58).

In most tissues examined except the spleen, expression of p21 is p53 independent (59, 60). Expression of p21 in tissues of the adult mouse is localized to terminally differentiating cells, and highest levels of p21 expression are present in tissues with high turnover rates, such as the skin and linings of the gastrointestinal tract (60–62). p21 expression is induced during the irreversible growth arrest of cellular senescence (9) and appears to be an important regulator of senescence in human fibroblasts (63). Overexpression of p21 led to the induction of a panel of genes expressed in different age-related diseases (64).

Disruption of the *p21* gene in the mouse did not lead to gross abnormalities, but studies with p21-deficient MEFs derived from these animals revealed an essential role for p21 in inducing growth arrest after DNA damage (20, 21). *In vivo* p21 has been shown to play roles in regulating renewal of keratinocytes (65) and hematopoietic cells (66). It plays a role in the control of T-cell proliferation, and female p21-deficient mice have decreased viability and develop a syndrome similar to human lupus (67). In the kidney, p21 appears to regulate the balance between hyperplasia and hypoplasia, and its disruption ameliorates progression to chronic renal failure after partial renal ablation (68). p21 induction was correlated with differentiation of muscle (59, 69), and embryos lacking both p21 and p57 have severe defects in the formation of skeletal muscle and altered lung development, suggesting redundant functions for p21 and p57 during development (70).

Aging studies in p21-deficient mice have demonstrated a role for p21 in tumor suppression, albeit a much weaker one than p53 (71). Mice deficient for p21 and the INK4 CKI p18 have increased incidence and progression of pituitary tumors, with most animals developing tumors by 1 year of age (72). Mice lacking p21 have been found to be more susceptible to chemically induced skin carcinoma (65, 73). Loss of p21 was associated with earlier development of mammary gland tumors, increased tumor multiplicity, and aggressiveness in MMTV/v-Ha-ras mice (74). p21 was also shown to synergize with Rb and INK4A/ARF to protect against tumor progression in mice (75, 76).

Activation of the tumor suppressor p53 generally leads to growth arrest and/or apoptosis, and its activities play important roles in preventing the development of cancer. Although p21 plays a critical role in inducing p53-dependent growth arrest after DNA damage, it is dispensable for p53-dependent apoptosis in the thymus (20) and intestinal crypts (21) of p21-deficient animals. p21 is also unnecessary for oncogene-induced apoptosis (77). Many recent studies have suggested that p21 is not only unessential but may actually act as an inhibitor of p53-dependent apoptosis. The effect of p21 expression on the regulation of apoptosis in a variety of systems and its possible ramifications in the treatment of cancer are discussed below.

## p21 Is a Negative Regulator of p53-dependent Apoptosis

In response to radiation and chemotherapy, p53 protein is stabilized and mediates apoptosis and cell cycle arrest. Whereas the mechanisms of p53-dependent apoptosis are not well understood, p53-dependent cycle arrest is primarily mediated by the CDK inhibitor p21. There is a mounting evidence that p21 is a major inhibitor of p53-dependent apoptosis (Fig. 1). It is not entirely clear how a cell chooses between apoptosis and p21-dependent cell cycle arrest after DNA damage and stabilization of p53, but often high levels of p21 expression mediate cell cycle arrest and protect from p53-dependent apoptosis.

Defects in p53-dependent induction of p21, repression of p53-dependent induction of p21 transcription, or caspase-mediated cleavage of p21 (Fig. 2) usually result in increased p53-dependent apoptosis. For example, human cancer cell lines treated with DNA-damaging agents undergo cell cycle arrest mediated by p21, followed by apoptosis after caspase 3-mediated cleavage of p21 (Ref. 78; Fig. 2*B*). Similarly, p53 point mutants that cannot transactivate the *p21* gene were more potent inducers of apoptosis than wild-type p53 (79–81). A431 cells expressing mutant p53 that cannot induce p21 undergo apoptosis after exposure to $\gamma$ or UV radiation, but induction of p21 by mimosine protects the same cells from apoptosis (82).

Triptolide, an immunosuppressive agent extracted from the Chinese herb *Tripterygium wilfordii,* enhanced apoptosis induced by the DNA-damaging drug Adriamycin (doxorubicin) by increasing expression of p53 protein and repressing p21 transcription (Fig. 1; Ref. 83). The recently discovered

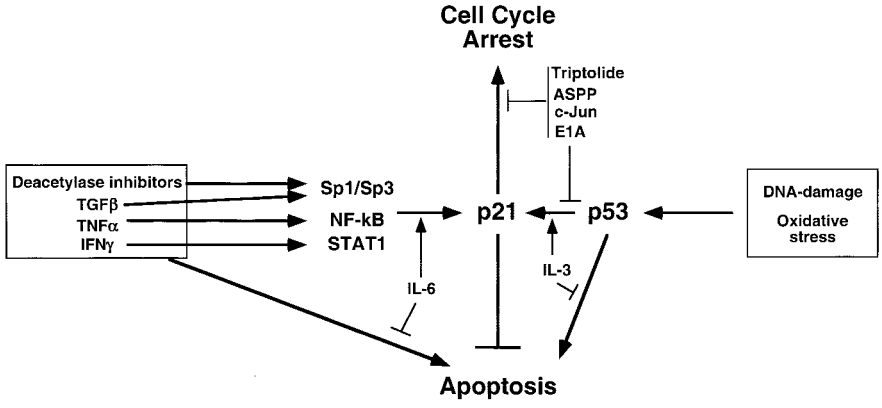


*Fig. 1*. p21 is a major inhibitor of p53-dependent and p53-independent apoptosis. DNA damage and oxidative stress ($H_2O_2$) activate two pathways, one involving p53-dependent apoptosis and the other involving p53-dependent activation of p21 that protects cells from apoptosis. Repression or elimination of p21 expression by E1A, triptolide, apoptosis-stimulating protein of p53 (*ASPP*), or c-Jun improves the apoptotic effect of p53, whereas induction of p21 by interleukin 3 (*IL-3*) suppresses p53-dependent apoptosis. TGF-$\beta$, TNF-$\alpha$, histone deacetylase inhibitors, IFN-$\gamma$, and interleukin 6 (*IL-6*) induce p53-independent activation of p21 via different transcription factors. p53-independent induction of p21 protects cells against p53-independent apoptosis.

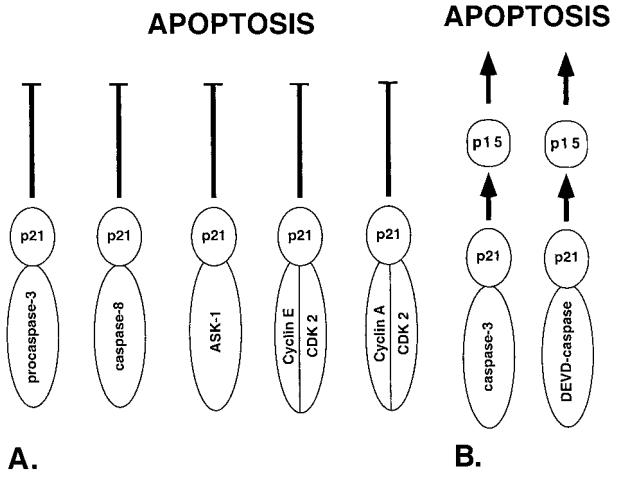


*Fig. 2*. Potential mechanisms for inhibition of apoptosis by p21. *A,* p21 may block apoptosis by interacting with proapoptotic molecules such as procaspase-3, caspase-8, and the kinase apoptosis signal-regulating kinase 1 (*ASK1*) or by inducing cell cycle arrest after binding to cyclin E/Cdk2 complexes or by binding to cyclin A/Cdk2 complexes. *B,* apoptosis in human endothelial cells after growth factor deprivation, hypoxia-induced apoptosis in cardiomyocytes, and apoptosis induced by zinc depletion in human epithelial lines is mediated by caspase-3-dependent cleavage of p21 and up-regulation of cyclin A/Cdk2 activity. Apoptosis induced by butyrate in colorectal cancer cells is mediated by DEVD-caspase-dependent cleavage of p21. Caspase-3- and DEVD-caspase-dependent cleavage of p21 results in the appearance of a p15 product and reduction of p21 activity.

family of apoptosis-stimulating protein of p53 proteins interacts with p53 and stimulates p53-dependent apoptosis. They enhance the binding and transactivation activities of p53 on the promoters of the proapoptotic genes *Bax* and *PIG-3* but not *p21* (Fig. 1; Ref. 84). Likewise, c-Jun negatively regulates the association of p53 with the p21 promoter and cells lacking c-Jun undergo prolonged p21-dependent cell cycle arrest in response to UV, whereas cells that express c-Jun undergo p53-dependent apoptosis (Fig. 1; Ref. 85).

Adriamycin induces apoptosis by p53-dependent and p53-independent mechanisms, and p53-dependent and p53-independent expression of p21 protects cells from Adriamycin-induced apoptosis (86). The HCT116 human colon carcinoma cell line undergoes cell cycle arrest after treatment with Adriamycin, but p21-null HCT116 cells undergo apoptosis after the same treatment (86, 87). Likewise, when HCT116 cells were infected with a recombinant adenovirus expressing wild-type p53, cells with intact *p21* and *14-3-3* genes underwent cell cycle arrest. In contrast, cells lacking either p21 or 14-3-3 underwent cell death, and double knockout cells died substantially more quickly than cells lacking only one of the two genes (87). Direct binding and inactivation of p21 by E1A leads to increased Cdk2 activity in DNA-damaged cells and results in induction of apoptosis after DNA damage in HCT116 cells (Fig. 1; Ref. 88). Interestingly, Adriamycin-induced apoptosis was inhibited by p21 with Cdk-inhibitory activity but not with p21 lacking this activity in the human p21 null colorectal cancer cell line (89).

Etoposide, a topoisomerase II inhibitor, is a cytotoxic drug that is commonly used for cancer chemotherapy. Exposure of human osteosarcoma cells with wild-type p53 to p21 overexpression before and during etoposide therapy-protected cells against etoposide-induced cell death (90). Irradiation of Baf-3 murine hematopoietic cells with wild-type p53 in the presence of interleukin 3 induces p21 and $G_1$ arrest (Fig. 1), whereas radiation in the absence of interleukin 3 results in much weaker induction of p21 and in apoptotic cell death (91). Furthermore, radiation therapy of xenograft tumors with intact *p21* genes was ineffective, whereas 18–38% of xenograft tumors from p21-deficient cells were cured (92), and p21 antisense therapy radiosensitized human colon cancer cells to apoptosis (93).

Similarly, oxidative stress ($H_2O_2$) activates two pathways: one involving p53-dependent apoptosis, and the other involving p53-dependent and -independent activation of p21 that protects cells from apoptosis (Ref. 94; Fig. 1). In macrophages, nitric oxide induces p53-dependent up-regulation of p21 and cell cycle arrest. Antisense p21 down-regulates p21 expression, abrogates $G_1$ arrest, and sensitize these cells toward apoptosis (95). In vascular smooth muscle cells, nitric oxide induces activation of p42/p44 mitogen-activated protein, p53-independent transactivation of p21, and cell cycle arrest (96). Hyperoxia leads to p53-dependent induction of p21 and cell cycle arrest in HCT116 colon carcinoma cell line (97) and to p53-dependent and -independent induction of p21 in lung cells (97). Hyperoxia in the absence of p21 or p53 led to apoptosis in HCT116 cells (97), suggesting that p53-

dependent induction of p21 after hyperoxia protects these cells against oxygen-induced toxicity (97). Survival of p21-deficient mice decreased 40% after hyperoxic lung injury, suggesting that p21 protects lung from oxidative stress (97).

Two types of colorectal cancer cell lines were identified with different responses to overexpression of exogenous p53. In response to p53, type A cell lines were found to undergo growth arrest, whereas type D cell lines underwent apoptosis (98). Inactivation of p21 by homologous recombination in A-type cells converted them to D-type cells, suggesting that p21 protects A-type cells from apoptosis. p53 overexpression has been found to be highly toxic for p21-deficient MEFs, but ectopic expression of exogenous p21 protects these cells from p53-induced apoptosis (99). These data suggest that p53-dependent induction of p21 negate apoptotic effect of p53. Repression or elimination of p21 expression by antisense p21, E1A, triptolide, or expression of p53 protein deficient in p21 transactivation improves apoptotic effect of p53, whereas overexpression of exogenous p21 or induction of endogenous p21 suppresses p53-dependent apoptosis (Fig. 1). Because p21 is one of the major direct targets of transactivation by p53, it acts as a major inhibitor of p53-dependent apoptosis.

### p21 Is a Negative Regulator of p53-independent Apoptosis

A number of different signals such as TGF-$\beta$, TNF-$\alpha$, histone deacetylase inhibitors, IFN-$\gamma$, and others may simultaneously induce p53-independent apoptosis and p53-independent transactivation of p21 (reviewed in Ref. 54; Fig. 1). In these cases, p21 protects cells against p53-independent apoptosis that is induced by these signals. TGF-$\beta$ inhibits cellular proliferation by cell cycle arrest of apoptosis. Induction of cell cycle arrest by TGF-$\beta$ is partly mediated via p53-independent transactivation of Cdk inhibitors p15, p21 (Fig. 1) and p27. Human gastric cells were initially arrested in $G_1$ by TGF-$\beta$ and then driven to apoptosis after caspase-3-induced cleavage of p21 (Fig. 2*B*) and p27 that led to aberrant activation of Cdk2 (100). In retinal endothelial cells, induction of p21 correlated with resistance to TGF-$\beta$-mediated apoptosis (101), suggesting that up-regulation of p21 inhibits TGF-$\beta$-induced apoptosis.

TNF-$\alpha$ and CD95 ligand are members of family of cytokines that are toxic to certain cancer cells. They interact with one or several cell surface receptors to trigger caspase activation and cytochrome *c* release. TNF-$\alpha$ induces apoptosis and p21 expression in highly malignant Ewing tumor cells by a p53-independent pathway (Fig. 1). Induction of p21 by TNF-$\alpha$ in these cells was NF-$\kappa$B dependent, and inhibition of NF-$\kappa$B activation led to sensitization of these cells to TNF-$\alpha$-induced apoptosis. Because overexpression of p21 in Ewing tumor cells protects them from TNF-$\alpha$-mediated apoptosis, authors of this work identified p21 as a mediator of the antiapoptotic effect of TNF-$\alpha$-induced NF-$\kappa$B in Ewing tumor cells (102). Similarly, TNF-$\alpha$ induces apoptosis and p21 expression in MCF-7 breast carcinoma cells (Fig. 1), and antisense p21 cDNA sensitizes these cells to TNF-$\alpha$-induced apoptosis (103). Differentiation-induced apoptosis of monocytes was found to be dependent on TNF-$\alpha$, and NF-$\kappa$B protects from TNF-$\alpha$-induced apoptosis during macrophage differentiation by up-regulation of p21 (104). In contrast, p21 proteolysis in ME-180 cells made them sensitive to TNF-$\alpha$-induced apoptosis (105). p21 antisense oligonucleotides sensitized human glioma cell lines to CD95 ligand-induced apoptosis (106) and sensitized the human promonocytic cell line to phorbol myristate acetate-induced (TNF-$\alpha$-dependent) apoptosis (104).

IFN-$\gamma$ induces p21 by a STAT1-dependent, p53-independent mechanism (Fig. 1; reviewed in Ref. 54), and p21 protects human colon adenocarcinoma cells from IFN-$\gamma$-induced apoptosis (107). IFN-$\gamma$ and decorin also activates macrophages and protects them from apoptosis by inducing p21 and cell cycle arrest at the $G_1$-S boundary (108, 109). Interleukin 6-type cytokines also induce the p21 promoter by a STAT-dependent mechanism, and p21 expression protects human osteoblastic cells from apoptosis (Fig. 1; Ref. 110).

Histone deacetylase inhibitors induce p53-independent expression of p21 via Sp1 binding sites in the p21 promoter (reviewed in Ref. 54) and p53-independent apoptosis and cell cycle arrest (Fig. 1). Trichostatin A induces p21, cell cycle arrest, and apoptosis in human gastric and oral carcinoma cell lines (111). Butyrate induces p21 and apoptosis in human colon tumor cell lines (112, 113), and the absence of p21 drastically augments butyrate-induced apoptosis in HCT116 colon carcinoma cell line (113). p21 was cleaved by DEVD-caspase in LIM1215 colorectal cancer cells that underwent butyrate-induced apoptosis (Fig. 2*B*; Ref. 112). A selected LIM1215 subclone that was resistant to butyrate-induced apoptosis lacked DEVD-caspase activity and characterized by failure of p21 cleavage. These cells showed enhanced p21 expression, suggesting that p21 protects them from butyrate-induced cell death (112). The hybrid polar compound and histone deacetylase inhibitor SAHA induced apoptosis in myelomonocytic leukemia cells and expression of p21 by the p53-independent pathway. Expression of antisense p21 increased SAHA-related lethality, indicating a protective role of p21 against SAHA-induced apoptosis (114). Similarly, histone deacetylase inhibitor azelaic bishydroxamic acid induces apoptosis and may up-regulate p21, which may reduce its cytotoxicity (115).

Levels of p21 often determine the cellular response to different drugs. For example, RKO human colorectal carcinoma cells normally undergo apoptosis in response to prostaglandin $A_2$, and these cells express low levels of p21 (116). In contrast, NIH 3T3 cells (117) and MCF-7 cells express high levels of p21 and undergo $G_1$ arrest in response to prostaglandin $A_2$ (116). Likewise, p53-independent up-regulation of p21 and $G_1$ arrest have been observed after treatment of MCF-7 cells with phenylacetate (118). In both cases, reduction of endogenous p21 expression in MCF-7 cells by antisense p21 RNA overexpression attenuates the growth arrest and promotes cell death. Inducible expression of exogenous p21 renders glioblastoma cells resistant to the chemotherapy agents BCNU and cisplatin (119) and provided protection against cytotoxic effects of Adriamycin and irradiation in p53-null lung cancer cell line H1299 (120). Inactivation of p53 sensitizes astrocytic U87MG glioma cells to BCNU by failure of p21 induction. In contrast, these cells with intact p53 function were resistant to BCNU and showed up-regulation

of p53, prolonged induction of p21, and S-phase arrest, suggesting that p53-dependent induction of p21 protects these cells from p53-independent apoptosis induced by BCNU (121). Soybean isoflavonoid genistein may induce apoptosis and possesses anticancer activity. However, in the presence of galectin-3, genistein induces p21 expression and $G_2$ arrest instead of apoptosis (122). p21 antisense-expressing human myelomonocytic leukemia U937 cells become susceptible to 1-$\beta$-D-arabinofuranosylcytosine-mediated mitochondrial dysfunction and apoptosis (123), and up-regulation of p21 blocks oxidative stress-induced apoptosis in human myeloma U266 cells that were originally apoptosis resistant (100).

Ataxia-telangiectasia is an autosomal recessive disorder linked to mutation of the *ATM* gene. Induction of p53 is impaired in ataxia-telangiecstasia cells, and cytotoxicity after ionizing radiation is p53 independent in ATM-deficient cells. Loss of p21 in the context of an ATM-deficient mouse leads to a delay in thymic lymphomagenesis and an increase in radiation sensitivity *in vivo,* suggesting again that p21 may act as an oncogene in these conditions (124). It has been suggested that normal function of p21 is to protect thymic tumor cells against apoptosis, and loss of p21 potentiates the apoptotic response (124). Similarly, p53-independent induction of B-lymphoma cell apoptosis by membrane IgM engagement is suppressed by simultaneous p53-independent induction of p21. Reduction of induced p21 protein levels in these cells by p21 antisense expression results in diminished $G_1$ arrest and increased apoptosis (125). Interestingly, loss of p21 also sensitizes solid tumors to radiation by an apoptosis-independent mechanism (126).

The gene encoding HER-2/neu, a $M_r$ 185,000 transmembrane receptor tyrosine kinase, was amplified or overexpressed in up to 30% of human breast carcinomas (127). Overexpression of the *HER-2/neu* gene correlated with poor prognosis and lower overall survival rate. HER-2/neu overexpression confers increased resistance of breast cancer cells to Taxol-induced apoptosis (128). Taxol is a highly effective antineoplastic agent for the treatment of metastatic breast cancers. It induces tubulin polymerization, microtubule formation (129), and apoptosis (130). p21-null cells were more sensitive to Taxol-induced cell death (131), and Taxol-induced apoptosis is inhibited by p21 (132, 133), probably, because of p21-dependent $G_2$ arrest (133). Overexpression of HER-2/neu in breast tumors induced transcriptional activation of the Cdk inhibitor p21 and activation of Akt kinase (128, 134). Akt physically associates with p21 and phosphorylates it at threonine 145, resulting in cytoplasmic localization of p21 (134). Cytoplasmic localization of p21 results in resistance of tumor cells to Taxol-induced apoptosis. Similar antiapoptotic activity of cytoplasmic p21 was observed in monocytic differentiation (135). Expression of antisense p21 in breast cancer cells with HER-2/neu overexpression sensitizes these cells to Taxol-induced apoptosis, and overexpression of HER-2/neu did not inhibit Taxol-induced apoptosis in $p21^{-/-}$ MEFs (136). These data suggested that overexpression of HER-2/neu in breast cancer cells prevents Taxol-induced apoptosis by transcriptional up-regulation, Akt-induced phosphorylation and translocation of p21 to cytoplasm (134). Akt also may phosphorylate p21 at serine 146 and increase its stability. It has been shown that glioblastoma cell lines with activated Akt display enhanced p21 stability and resistance to Taxol-mediated apoptosis (137).

p21 also inhibits apoptosis during cell differentiation. For example, insulin-like growth factor I promotes muscle cell survival as an early event in differentiation through a pathway that involves Akt-dependent induction of p21 (138). Growth factor withdrawal from murine C2C12 myoblasts induces differentiation and extensive cell death. During differentiation, the appearance of an apoptosis-resistant phenotype correlates with expression of p21, and forced expression of p21 blocks apoptosis during myocyte differentiation (138, 139). Antisense oligonucleotides against p21 block cell cycle withdrawal and enhance myocyte cell death (138, 140). Similarly, the treatment of differentiating neuroblastoma cells with p21 antisense oligonucleotides leads to a decrease of both expression of p21 protein and cell survival (141).

### Potential Mechanisms Leading to the Inhibition of Apoptosis by p21

The mechanisms by which p21 can prevent cells from undergoing apoptosis are not well understood. One mechanism is assumed to involve p21-dependent cell cycle arrest (often at $G_2$-M) that would permit repair or prevent DNA damage (Fig. 2*A*). For example, adeno-associated virus selectively induces apoptosis in cells that lack p53 or p21. Cells with intact p53 and p21 activities are not killed but undergo arrest at the $G_2$ phase of the cell cycle, which is characterized by an increase in p53 activity and p21 levels (142). Cells with normal p53 and p21 pause at $G_2$ to eliminate the adeno-associated virus genome and then start cell division again. Cells without p53/p21 could not maintain their $G_2$ phase arrest and began catastrophic nuclear division that led to cell death (143). In this case, the ability of p21 to mediate p53-dependent $G_2$ arrest after DNA damage protects cells from apoptosis. Similarly, galectin-3-mediated, genistein-induced $G_2$ arrest was associated with up-regulation of p21 and inhibition of genistein-stimulated apoptosis (122). In accordance with the same mechanism, protection from paclitaxel- or cisplatin-induced apoptosis by overexpression of p21 was based on functional Rb status and ability of p21 to induce cell cycle arrest (144).

Another distinct mechanism is linked to the ability of p21 to bind and inactivate cyclin A/Cdk2 complexes (Fig. 2*A*). It has been shown that caspase-3-mediated cleavage of p21 is an important mechanism for death-associated cyclin A/Cdk2 activation in different cell types (Fig. 2*B*; Refs. 145–148). Apoptosis in human endothelial cells after growth factor deprivation (145), hypoxia-induced apoptosis in cardiomyocytes (148), apoptosis induced by zinc depletion in human epithelial lines (112), and apoptosis induced by other stimuli (146, 147) were mediated by caspase-3 cleavage of p21 and subsequent up-regulation of cyclin A/Cdk 2 activity. Caspase-dependent Cdk2 activity is a requisite effector of apoptotic death, and it may be necessary for death-associated chromatin condensation, cell shrinking, and loss of adhesion to substrate (149). Interestingly, p21 was overexpressed and associated with cyclin A/Cdk2 complexes in

human T-cell lymphotropic virus-1-infected cells that were resistant to DNA damage-induced apoptosis (150). It remains to be seen whether cyclin A/Cdk2 activity is a general requirement for the execution of cell death and whether p21 generally inhibits this activity.

Recent data also point to more direct mechanisms by which p21 may inhibit cell death. These include inhibition of initiator caspase cleavage by p21 (Fig. 2*A*; Ref. 151), p21 interaction with procaspase 3 leading to resistance to Fas-mediated cell death (Fig. 2*A*; Ref. 152), and stabilization of the apoptotic inhibitor protein c-IAP1 (153). Overexpression of p21 completely blocked DR4 TRAIL receptor cytoplasmic domain (CD) induced cleavage of caspase-8 and DR4-CD-induced apoptosis, and this activity resides within 91 amino acids of the $NH_2$ terminus of p21 protein (Fig. 2*A*; Ref. 151). p21 interacts with procaspase-3 through the $NH_2$ terminus and suppresses its activation by the masking the serine proteinase-cleaving site (Fig. 2*A*; Refs. 152, 154). Procaspase-3-p21 complex formation occurred in mitochondria and required phosphorylation of p21 by protein kinase A (155). Alternatively, phosphorylation of p21 at threonine 145 by Akt may cause p21 relocalization to the cytoplasm (134), where it may form a complex with apoptosis signal-regulating kinase 1 (Fig. 2*A*; Ref. 135) and inhibit stress-activated mitogen-activated protein kinase cascade and cell death induced by TNF-$\alpha$ and other stimuli.

### Conundrum: p21 Induces Apoptosis

A variety of types of cellular stress lead to induction of p21 expression by both p53-dependent and -independent mechanisms. As discussed above, p21-induced growth arrest can protect cells and inhibit apoptosis. However, a number of reports also suggest that p21 possesses proapoptotic functions under certain conditions in specific systems. Overexpression of p21 in thymocytes led to hypersensitivity to p53-dependent cell death in response to ionizing radiation and UV but not to dexamethasone in transgenic animals (156). Overexpression of p21 in C3 (1) SV40 T-antigen mammary tumor cells deficient for p53 activity increased apoptosis (157). Overexpression of p21 has been shown to enhance the apoptotic response to the chemotherapeutic agent cisplatin in glioma (158) and ovarian carcinoma (159) cell lines. Cisplatin induces p53-dependent apoptosis of U87-MG glioma cells and p53-independent apoptosis of GB-1 glioma cells. Overexpression of p21 in U87-MG cells led to growth arrest and an apparent apoptotic morphology, and clonal U87-MG p21-overexpressing cell lines were not obtained. However, clones of GB-1 cells overexpressing p21 were derived, and these clones displayed increased sensitivity to cisplatin (158). Overexpression of p21 in the p53-deficient human ovarian carcinoma cell lines SKOV3 and OVCAR3 also led to increased apoptosis in response to cisplatin treatment (159).

Overexpression of p21 also promoted $C_6$-ceramide-induced apoptosis in the p53-deficient Hep3B human hepatoma cell line. In these cells, overexpression of p21 resulted in induction of the proapoptotic protein Bax modulating the molecular ratio of Bcl-2:Bax in these cells (160). Similarly, p21 and Bax were up-regulated in Hep3B cells undergoing retinoic acid-induced apoptosis, and antisense oligonucleotides complementary to p21 mRNA significantly rescued retinoic acid-induced apoptosis (161). A protein tyrosine kinase inhibitor, the isoflavonoid genistein, induced p53-independent induction of p21 and Bax and apoptosis in the breast cancer cell line MDA-MB-231 that contains mutant p53 (162). In these cases, p21 expression correlated with induction of the proapoptotic Bax protein.

Introduction of a p21-expressing adenovirus into a panel of p53-deficient cervical cancer cell lines resulted in both growth inhibition and apoptosis. Apoptosis was induced in experimental cervical tumors established with these cell lines in nude mice after injection of an adenovirus expressing a p21 sense RNA but not p21 antisense RNA (163). In these experiments, overexpression of the antiapoptotic protein Bcl-2 was unable to overcome apoptosis induced by p21. Introduction of adenovirally transduced p21 also lead to apoptosis in esophageal cancer cells (164). It remains to be seen whether there is cell type specificity in the apoptotic response to p21 overexpression, because no apoptosis was observed when an p21 adenovirus was introduced into melanoma cells (165).

p21 may play an active role in apoptosis induced by activation of members of the TNF family of death receptors. p53-independent induction of p21 expression and apoptosis was observed after ligation of CD95/FAS on T lymphocytes. A reduction in Fas-dependent apoptosis was seen in thymocytes deficient for p21 when compared with wild-type control thymocytes (166). The peroxisome proliferator-activated receptor-$\gamma$ agonist pioglitazone enhanced the sensitivity of NCI-H727 cells to apoptosis induced by the TRAIL ligand. Pioglitazone treatment also led to induction of p21, and adenovirus-mediated overexpression of p21 in these cells significantly enhanced the level of TRAIL-induced apoptosis (167).

Overexpression of human RAD50, a DNA recombination repair gene, causes cell death. Introduction of hRAD50 into HCT116 colon carcinoma cells lacking p21 caused no cytotoxicity, whereas there was a significant decrease in cell survival in the parental HCT16 cells transfected with the hRAD50 construct (168). Treatment of HCT116 cells with the antioxidants PDTC and vitamin E led to apoptosis and increased sensitivity of HCT116-derived xenograft tumors to the chemotherapeutic agent 5-FU and p53-independent induction of p21. A significant decrease in apoptosis was detected in xenograft tumors derived from HCT116 cells lacking p21 in response to PDTC and 5-FU (169). In this model, p21 appears important for antioxidant-mediated toxicity. However, although PDTC enhanced the apoptotic effects of 5-FU in the HCT116 colon carcinoma cell line, it appeared to protect cells from 5-FU-induced apoptosis in the normal mouse colon *in vivo* (170). The antioxidant resveratrol induced p21 in a p53-independent manner and apoptosis in the A431 cell line (171).

In most of the studies described above, data are presented that show that targeted overexpression of p21 increases apoptosis, or disruption of p21 function leads to a decrease in apoptosis. In most cases, the systems used lack functional p53. The mechanisms by which p21 may promote apoptosis are not currently understood but could be related to its ability

to interact with and possibly regulate components of the DNA repair machinery. Overall, the number of reports indicating an antiapoptotic role for p21 far outnumbers those suggesting it has apoptosis-promoting abilities.

## Conclusions

The Cdk inhibitor p21 is often responsible for stress-induced p53-dependent and p53-independent cell cycle arrest. Cell cycle arrest permits cells to pause and to repair damage and then to continue cell division. On one hand, the function of p21 to inhibit cell proliferation may contribute to its ability to act as tumor suppressor. On the other hand, the capacity of p21 to induce cell cycle arrest after stress can protect cells from stress-induced apoptosis. Antiapoptotic activity of p21 may contribute to its potential to act as an oncogene. Similar to the growth-promoting oncoproteins Myc and E2F1 that have an "antagonistic duality" because they possess the ability to promote apoptosis (anticancer) to counter to their proliferative activity (procancer; Ref. 172), the growth-arresting protein p21 has an "antagonistic duality" in that it often inhibits apoptosis (procancer) to counter to its antiproliferative effects (anticancer). Six years ago when we addressed the role of p21 in cancer in our previous review (173), p21 was perceived mainly as an anticancer protein because of its antiproliferative effects. Now its role in human cancer has become more controversial because it may also possess antiapoptotic (procancer) capabilities.

Anticancer drugs kill cancer cells by inducing p53-dependent and p53-independent apoptosis, and p21 protects cells from anticancer drug-induced apoptosis. Because loss of p21 usually increases sensitivity of tumor cells to apoptosis induced by different chemotherapeutic agents, small molecules that eliminate p21 expression may improve the action of anticancer drugs. Therefore, functional p21 may suppress tumor growth in the organism, but at the same time elimination of p21 may be beneficial during chemotherapy.

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## References

1. Sherr, C. J. Mammalian G1 cyclins. Cell, *73:* 1059–1065, 1993.

2. Morgan, D. O. Principles of CDK regulation. Nature (Lond.), *374:* 131–134, 1995.

3. Ekholm, S. V., and Reed, S. I. Regulation of $G_1$ cyclin-dependent kinases in the mammalian cell cycle. Curr. Opin. Cell Biol., *12:* 676–684, 2000.

4. Sherr, C. J., and Roberts, J. M. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev., *13:* 1501–1512, 1999.

5. Sherr, C. J. The Pezcoller lecture: cancer cell cycles revisited. Cancer Res., *60:* 3689–3695, 2000.

6. El-Deiry, W., Tokino, T., Velculescu, V., Levy, D., Parsons, R., Trent, J., Lin, D., Mercer, W. E., Kinzler, K., and Vogelstein, B. WAF1, a potential mediator of p53 tumor suppression. Cell, *75:* 817–825, 1993.

7. Harper, J. W., Adami, G., Wei, N., Keyomarsi, K., and Elledge, S. The p21 cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell, *75:* 805–816, 1993.

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

9. Noda, A., Ning, Y., Venable, S. F., Pereira-Smith, O. M., and Smith, J. R. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. Exp. Cell Res., *211:* 90–98, 1994.

10. Polyak, K., Kato, J. Y., Solomon, M. J., Sherr, C. J., Massagué, J., Roberts, J. M., and Koff, A. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-$\beta$ and contact inhibition to cell cycle arrest. Genes Dev., *8:* 9–22, 1994.

11. Toyoshima, H., and Hunter, T. p27, a novel inhibitor of G1 cyclin/cdk protein kinase activity, is related to p21. Cell, *78:* 67–74, 1994.

12. Lee, M. H., Reynisdottir, I., and Massagué, J. Cloning of p57KIP2, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. Genes Dev., *9:* 639–649, 1995.

13. Matsuoka, S., Edwards, M., Bai, C., Parker, S., Zhang, P., Baldini, A., Harper, J., and Elledge, S. p57, a structurally distinct member of the p21 cdk inhibitor family, is a candidate tumor suppressor gene. Genes Dev., *9:* 650–662, 1995.

14. Polyak, K., Lee, M. H., Erdjument-Bromage, H., Koff, A., Roberts, J. M., Tempst, P., and Massagué, J. Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. Cell, *78:* 59–66, 1994.

15. Chen, J., Jackson, P. K., Kirschner, M. W., and Dutta, A. Separate domains of p21 involved in the inhibition of Cdk kinase and PCNA. Nature (Lond.), *374:* 386–388, 1995.

16. Luo, Y., Hurwitz, J., and Massagué, J. Cell-cycle inhibition by independent CDK and PCNA binding domains in p21. Nature (Lond.), *375:* 159–161, 1995.

17. Harper, J. W., Elledge, S. J., Keyomarsi, K., Dynlacht, B., Tsai, L., Zhang, P., Dobtowolski, S., Bai, C., Connell-Crowley, L., Swindell, E., Fox, M. P., and Wei, N. Inhibition of cyclin dependent kinases by p21. Mol. Biol. Cell, *6:* 387–400, 1995.

18. Zhang, H., Xiong, Y., and Beach, D. Proliferating cell nuclear antigen and p21 are components of multiple cell cycle kinase complexes. Mol. Biol. Cell, *4:* 897–906, 1993.

19. Dulic, V., Kaufman, W. K., Wilson, S. J., Tlsty, T. D., Lees, E., Harper, J. W., Elledge, S. J., and Reed, S. I. p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. Cell, *76:* 1013–1023, 1994.

20. Deng, C., Zhang, P., Harper, J. W., Elledge, S. J., and Leder, P. Mice lacking p21ClP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. Cell, *82:* 675–684, 1995.

21. Brugarolas, J., Chadrasekaran, C., Gordon, J., Beach, D., Jacks, T., and Hannon, G. Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature (Lond.), *377:* 552–557, 1995.

22. Niculescu, A. R., Chen, X., Smeets, M., Hengst, L., Prives, C., and Reed, S. I. Effects of p21(Cip1/Waf1) at both the G1/S and the G2/M cell cycle transitions: pRb is a critical determinant in blocking DNA replication and in preventing endoreduplication [published erratum appears in Mol. Cell. Biol., *18:* 1763, 1998]. Mol. Cell. Biol., *18:* 629–643, 1998.

23. Ogryzko, V. V., Wong, P., and Howard, B. H. WAF1 retards S-phase progression primarily by inhibition of cyclin-dependent kinases. Mol. Cell. Biol., *17:* 4877–4882, 1997.

24. Zhang, H., Hannon, G., and Beach, D. p21-containing cyclin kinases exist in both active and inactive states. Genes Dev., *8:* 1750–1758, 1994.

25. Soos, T. J., Kiyokawa, H., Yan, J. S., Rubin, M. S., Giordano, A., DeBlasio, A., Bottega, S., Wong, B., Mendelsohn, J., and Koff, A. Formation of p27-CDK complexes during the human mitotic cell cycle. Cell Growth Differ., *7:* 135–146, 1996.

26. Blain, S. W., Montalvo, E., and Massagué, J. Differential interaction of the cyclin-dependent kinase (Cdk) inhibitor p27Kip1 with cyclin A-Cdk2 and cyclin D2-Cdk4. J. Biol. Chem., *272:* 25863–25872, 1997.

27. LaBaer, J., Garrett, M. D., Stevenson, L. F., Slingerland, J. M., Sandhu, C., Chou, H. S., Fattaey, A., and Harlow, E. New functional activities for the p21 family of CDK inhibitors. Genes Dev., *11:* 847–862, 1997.

28. Besson, A., and Yong, V. W. Involvement of p21(Waf1/Cip1) in protein kinase Cα-induced cell cycle progression. Mol. Cell. Biol., *20:* 4580–4590, 2000.

29. Weiss, R. H., Joo, A., and Randour, C. p21(Waf1/Cip1) is an assembly factor required for platelet-derived growth factor-induced vascular smooth muscle cell proliferation. J. Biol. Chem., *275:* 10285–10290, 2000.

30. Cheng, M., Olivier, P., Diehl, J. A., Fero, M., Roussel, M. F., Roberts, J. M., and Sherr, C. J. The p21(Cip1) and p27(Kip1) CDK "inhibitors" are essential activators of cyclin D-dependent kinases in murine fibroblasts. EMBO J., *18:* 1571–1583, 1999.

31. Bagui, T. K., Jackson, R. J., Agrawal, D., and Pledger, W. J. Analysis of cyclin D3-cdk4 complexes in fibroblasts expressing and lacking p27(kip1) and p21(cip1). Mol. Cell. Biol., *20:* 8748–8757, 2000.

32. Cheng, M., Sexl, V., Sherr, C. J., and Roussel, M. F. Assembly of cyclin D-dependent kinase and titration of p27Kip1 regulated by mitogen-activated protein kinase kinase (MEK1). Proc. Natl. Acad. Sci. USA, *95:* 1091–1096, 1998.

33. Perez-Roger, I., Kim, S. H., Griffiths, B., Sewing, A., and Land, H. Cyclins D1 and D2 mediate myc-induced proliferation via sequestration of p27(Kip1) and p21(Cip1). EMBO J., *18:* 5310–5320, 1999.

34. Bouchard, C., Thieke, K., Maier, A., Saffrich, R., Hanley-Hyde, J., Ansorge, W., Reed, S., Sicinski, P., Bartek, J., and Eilers, M. Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. EMBO J., *18:* 5321–5333, 1999.

35. McConnell, B. B., Gregory, F. J., Stott, F. J., Hara, E., and Peters, G. Induced expression of p16(INK4a) inhibits both CDK4- and CDK2-associated kinase activity by reassortment of cyclin-CDK-inhibitor complexes. Mol. Cell. Biol., *19:* 1981–1989, 1999.

36. Tong, W., and Pollard, J. W. Genetic evidence for the interactions of cyclin D1 and p27(Kip1) in mice. Mol. Cell. Biol., *21:* 1319–1328, 2001.

37. Geng, Y., Yu, Q., Sicinska, E., Das, M., Bronson, R. T., and Sicinski, P. Deletion of the *p27Kip1* gene restores normal development in cyclin D1-deficient mice. Proc. Natl. Acad. Sci. USA, *98:* 194–199, 2001.

38. Tsutsui, T., Hesabi, B., Moons, D. S., Pandolfi, P. P., Hansel, K. S., Koff, A., and Kiyokawa, H. Targeted disruption of CDK4 delays cell cycle entry with enhanced p27(Kip1) activity. Mol. Cell. Biol., *19:* 7011–7019, 1999.

39. Xiong, Y., Zhang, H., and Beach, D. D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. Cell, *71:* 505–514, 1992.

40. Tsurimoto, T. PCNA binding proteins. Front. Biosci., *4:* D849–D858, 1999.

41. Warbrick, E. The puzzle of PCNA's many partners. Bioessays, *22:* 997–1006, 2000.

42. Waga, S., Hannon, G., Beach, D., and Stillman, B. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. Nature (Lond.), *369:* 574–578, 1994.

43. Flores-Rozas, H., Kelman, Z., Dean, F. B., Pan, Z. Q., Harper, J. W., Elledge, S. J., O'Donnell, M. O., and Hurwitz, J. Cdk-interacting protein 1 directly binds with proliferating cell nuclear antigen and inhibits DNA replication catalyzed by the DNA polymer as delta holoenzyme. Proc. Natl. Acad. Sci. USA, *91:* 8655–8659, 1994.

44. Gibbs, E., Kelman, Z., Gulbis, J. M., O'Donnell, M., Kuriyan, J., Burgers, P., and Hurwitz, J. The influence of the proliferating cell nuclear antigen-interacting domain of p21(CIP1) on DNA synthesis catalyzed by the human and *Saccharomyces cerevisiae* polymerase delta holoenzymes. J. Biol. Chem., *272:* 2373–2381, 1997.

45. McDonald, E. R., Wu, G. S., Waldman, T., and El-Deiry, W. S. Repair defect in p21 WAF1/CIP1 −/− human cancer cells. Cancer Res., *56:* 2250–2255, 1996.

46. Sheikh, M. S., Chen, Y. Q., Smith, M. L., and Fornace, A. J., Jr. Role of p21Waf1/Cip1/Sdi1 in cell death and DNA repair as studied using a tetracycline-inducible system in p53-deficient cells. Oncogene, *14:* 1875–1882, 1997.

47. Li, R., Waga, S., Hannon, G., Beach, D., and Stillman, B. Differential effects by the p21 CDK inhibitor on PCNA dependent DNA replication and repair. Nature, *371:* 534–537, 1994.

48. Shivji, M. K., Grey, S. J., Strausfeld, U. P., Wood, R. D., and Blow, J. J. Cip1 inhibits DNA replication but not PCNA-dependent nucleotide excision-repair. Curr. Biol., *4:* 1062–1068, 1994.

49. Adimoolam, S., Lin, C. X., and Ford, J. M. The p53-regulated cyclin-dependent kinase inhibitor, p21 (cip1, waf1, sdi1), is not required for global genomic and transcription-coupled nucleotide excision repair of UV-induced DNA photoproducts. J. Biol. Chem., *276:* 25813–25822, 2001.

50. Pan, Z. Q., Reardon, J. T., Li, L., Flores-Rozas, H., Legerski, R., Sancar, A., and Hurwitz, J. Inhibition of nucleotide excision repair by the cyclin-dependent kinase inhibitor p21. J. Biol. Chem., *270:* 22008–22016, 1995.

51. Cooper, M. P., Balajee, A. S., and Bohr, V. A. The C-terminal domain of p21 inhibits nucleotide excision repair *in vitro* and *in vivo*. Mol. Biol. Cell, *10:* 2119–2129, 1999.

52. Umar, A., Buermeyer, A. B., Simon, J. A., Thomas, D. C., Clark, A. B., Liskay, R. M., and Kunkel, T. A. Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. Cell, *87:* 65–73, 1996.

53. Tom, S., Ranalli, T. A., Podust, V. N., and Bambara, R. A. Regulatory roles of p21 and apurinic/apyrimidinic endonuclease 1 in base excision repair. J. Biol. Chem., *276:* 48781–48789, 2001.

54. Gartel, A. L., and Tyner, A. L. Transcriptional regulation of the *p21(WAF1/CIP1)* gene. Exp. Cell Res., *246:* 280–289, 1999.

55. El-Deiry, W. S., Tokino, T., Waldman, T., Oliner, J. D., Velculscu, V. E., Burrel, M., Hill, D. E., Healy, E., Rees, J. L., Hamilton, S. R., Kinzler, K. W., and Vogelstein, B. Topological control of p21 (WAF1/CIP1) expression in normal and neoplastic tissues. Cancer Res., *55:* 2910–2919, 1995.

56. Maki, C. G., and Howley, P. M. Ubiquitination of p53 and p21 is differentially affected by ionizing and UV radiation. Mol. Cell. Biol., *17:* 355–363, 1997.

57. Sheaff, R. J., Singer, J. D., Swanger, J., Smitherman, M., Roberts, J. M., and Clurman, B. E. Proteasomal turnover of p21Cip1 does not require p21Cip1 ubiquitination. Mol. Cell, *5:* 403–410, 2000.

58. Touitou, R., Richardson, J., Bose, S., Nakanishi, M., Rivett, J., and Allday, M. J. A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8α subunit of the 20S proteasome. EMBO J., *20:* 2367–2375, 2001.

59. Parker, S., Eichele, G., Zhang, P., Rawls, A., Sands, A., Bradley, A., Olson, E., Harper, J., and Elledge, S. p53-independent expression of p21 in muscle and other terminally differentiating cells. Science (Wash. DC), *267:* 1024–1027, 1995.

60. Macleod, K., Sherry, N., Hannon, G., Beach, D., Tokino, T., Kinzler, K., Vogelstein, B., and Jacks, T. p53-dependent and independent expression of p21 during cell growth, differentiation, and DNA damage. Genes Dev., *9:* 935–944, 1995.

61. Huppi, K., Siwarski, D., Dosik, J., Michieli, P., Chedid, M., Reed, S., Mock, B., Givol, D., and Mushinski, J. F. Molecular cloning, sequencing, chromosomal location and expression of mouse p21 (Waf1). Oncogene, *9:* 3017–3020, 1994.

62. Gartel, A. L., Serfas, M. S., Gartel, M., Goufman, E., Wu, G. S., el-Deiry, W. S., and Tyner, A. L. p21 (WAF1/CIP1) expression is induced in newly nondividing cells in diverse epithelia and during differentiation of the Caco-2 intestinal cell line. Exp. Cell Res., *227:* 171–181, 1996.

63. Brown, J. P., Wei, W., and Sedivy, J. M. Bypass of senescence after disruption of *p21CIP1/WAF1* gene in normal diploid human fibroblasts. Science (Wash. DC), *277:* 831–834, 1997.

64. Chang, B. D., Watanabe, K., Broude, E. V., Fang, J., Poole, J. C., Kalinichenko, T. V., and Roninson, I. B. Effects of p21Waf1/Cip1/Sdi1 on cellular gene expression: implications for carcinogenesis, senescence, and age-related diseases. Proc. Natl. Acad. Sci. USA, *97:* 4291–4296, 2000.

65. Topley, G. I., Okuyama, R., Gonzales, J. G., Conti, C., and Dotto, G. P. p21(WAF1/Cip1) functions as a suppressor of malignant skin tumor formation and a determinant of keratinocyte stem-cell potential. Proc. Natl. Acad. Sci. USA, *96:* 9089–9094, 1999.

66. Cheng, T., Rodrigues, N., Shen, H., Yang, Y., Dombkowski, D., Sykes, M., and Scadden, D. T. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science (Wash. DC), *287:* 1804–1808, 2000.

67. Balomenos, D., Martin-Caballero, J., Garcia, M. I., Prieto, I., Flores, J. M., Serrano, M., and Martinez, A. C. The cell cycle inhibitor p21 controls T-cell proliferation and sex-linked lupus development. Nat. Med., *6:* 171–176, 2000.

68. Megyesi, J., Price, P. M., Tamayo, E., and Safirstein, R. L. The lack of a functional *p21(WAF1/CIP1)* gene ameliorates progression to chronic renal failure. Proc. Natl. Acad. Sci. USA, *96:* 10830–10835, 1999.

69. Halevy, O., Novitch, B. G., Spicer, D. B., Skapek, S. X., Rhee, J., Hannon, G. J., Beach, D., and Lassar, A. B. Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD. Science (Wash. DC), *267:* 1018–1021, 1995.

70. Zhang, P., Wong, C., Liu, D., Finegold, M., Harper, J. W., and Elledge, S. J. p21(CIP1) and p57(KIP2) control muscle differentiation at the myogenin step. Genes Dev., *13:* 213–224, 1999.

71. Martin-Caballero, J., Flores, J. M., Garcia-Palencia, P., and Serrano, M. Tumor susceptibility of p21(Waf1/Cip1)-deficient mice. Cancer Res., *61:* 6234–6238, 2001.

72. Franklin, D. S., Godfrey, V. L., O'Brien, D. A., Deng, C., and Xiong, Y. Functional collaboration between different cyclin-dependent kinase inhibitors suppresses tumor growth with distinct tissue specificity. Mol. Cell. Biol., *20:* 6147–6158, 2000.

73. Philipp, J., Vo, K., Gurley, K. E., Seidel, K., and Kemp, C. J. Tumor suppression by p27Kip1 and p21Cip1 during chemically induced skin carcinogenesis. Oncogene, *18:* 4689–4698, 1999.

74. Adnane, J., Jackson, R. J., Nicosia, S. V., Cantor, A. B., Pledger, W. J., and Sebti, S. M. Loss of p21WAF1/CIP1 accelerates Ras oncogenesis in a transgenic/knockout mammary cancer model. Oncogene, *19:* 5338–5347, 2000.

75. Brugarolas, J., Bronson, R. T., and Jacks, T. p21 is a critical CDK2 regulator essential for proliferation control in Rb-deficient cells. J. Cell Biol., *141:* 503–514, 1998.

76. Paramio, J. M., Segrelles, C., Ruiz, S., Martin-Caballero, J., Page, A., Martinez, J., Serrano, M., and Jorcano, J. L. The ink4a/arf tumor suppressors cooperate with p21cip1/waf in the processes of mouse epidermal differentiation, senescence, and carcinogenesis. J. Biol. Chem., *276:* 44203–44211, 2001.

77. Attardi, L. D., Lowe, S. W., Brugarolas, J., and Jacks, T. Transcriptional activation by p53, but not induction of the *p21* gene, is essential for oncogene-mediated apoptosis. EMBO J., *15:* 3693–3701, 1996.

78. Zhang, Y., Fujita, N., and Tsuruo, T. Caspase-mediated cleavage of p21Waf1/Cip1 converts cancer cells from growth arrest to undergoing apoptosis. Oncogene, *18:* 1131–1138, 1999.

79. Kaneuchi, M., Yamashita, T., Shindoh, M., Segawa, K., Takahashi, S., Furuta, I., Fujimoto, S., and Fujinaga, K. Induction of apoptosis by the p53–273L (Arg→Leu) mutant in HSC3 cells without transactivation of p21Waf1/Cip1/Sdi1 and bax. Mol. Carcinog., *26:* 44–52, 1999.

80. Okaichi, K., Wang, L. H., Sasaki, J., Saya, H., Tada, M., and Okumura, Y. A point mutation of human p53, which was not detected as a mutation by a yeast functional assay, led to apoptosis but not p21Waf1/Cip1/Sdi1 expression in response to ionizing radiation in a human osteosarcoma cell line, Saos-2. Int. J. Radiat. Oncol. Biol. Phys., *45:* 975–980, 1999.

81. Kokontis, J. M., Wagner, A. J., O'Leary, M., Liao, S., and Hay, N. A transcriptional activation function of p53 is dispensable for and inhibitory of its apoptotic function. Oncogene, *20:* 659–668, 2001.

82. Bissonnette, N., and Hunting, D. J. p21-induced cycle arrest in G1 protects cells from apoptosis induced by UV-irradiation or RNA polymerase II blockage. Oncogene, *16:* 3461–3469, 1998.

83. Chang, W. T., Kang, J. J., Lee, K. Y., Wei, K., Anderson, E., Gotmare, S., Ross, J. A., and Rosen, G. D. Triptolide and chemotherapy cooperate in tumor cell apoptosis. A role for the p53 pathway. J. Biol. Chem., *276:* 2221–2227, 2001.

84. Samuels-Lev, Y., O'Connor, D. J., Bergamaschi, D., Trigiante, G., Hsieh, J. K., Zhong, S., Campargue, I., Naumovski, L., Crook, T., and Lu, X. ASPP proteins specifically stimulate the apoptotic function of p53. Mol. Cell, *8:* 781–794, 2001.

85. Shaulian, E., Schreiber, M., Piu, F., Beeche, M., Wagner, E. F., and Karin, M. The mammalian UV response: c-Jun induction is required for exit from p53-imposed growth arrest. Cell, *103:* 897–907, 2000.

86. Bunz, F., Hwang, P. M., Torrance, C., Waldman, T., Zhang, Y., Dillehay, L., Williams, J., Lengauer, C., Kinzler, K. W., and Vogelstein, B. Disruption of p53 in human cancer cells alters the responses to therapeutic agents. J. Clin. Investig., *104:* 263–269, 1999.

87. Chan, T. A., Hwang, P. M., Hermeking, H., Kinzler, K. W., and Vogelstein, B. Cooperative effects of genes controlling the G(2)/M checkpoint. Genes Dev., *14:* 1584–1588, 2000.

88. Chattopadhyay, D., Ghosh, M. K., Mal, A., and Harter, M. L. Inactivation of p21 by E1A leads to the induction of apoptosis in DNA- damaged cells. J. Virol., *75:* 9844–9856, 2001.

89. Lu, Y., Tatsuka, M., Takebe, H., and Yagi, T. Involvement of cyclin-dependent kinases in doxorubicin-induced apoptosis in human tumor cells. Mol. Carcinog., *29:* 1–7, 2000.

90. Prabhu, N. S., Somasundaram, K., Tian, H., Enders, G. H., Satyamoorthy, K., Herlyn, M., and El-Deiry, W. S. The administration schedule of cyclin-dependent kinase inhibitor gene therapy and etoposide chemotherapy is a major determinant of cytotoxicity. Int. J. Oncol., *15:* 209–216, 1999.

91. Canman, C. E., Gilmer, T. M., Coutts, S. B., and Kastan, M. B. Growth factor modulation of p53-mediated growth arrest *versus* apoptosis. Genes Dev., *9:* 600–611, 1995.

92. Waldman, T., Zhang, Y., Dillehay, L., Yu, J., Kinzler, K., Vogelstein, B., and Williams, J. Cell-cycle arrest *versus* cell death in cancer therapy. Nat. Med., *3:* 1034–1036, 1997.

93. Tian, H., Wittmack, E. K., and Jorgensen, T. J. p21WAF1/CIP1 antisense therapy radiosensitizes human colon cancer by converting growth arrest to apoptosis. Cancer Res., *60:* 679–684, 2000.

94. Migliaccio, E., Giorgio, M., Mele, S., Pelicci, G., Reboldi, P., Pandolfi, P. P., Lanfrancone, L., and Pelicci, P. G. The p66shc adaptor protein controls oxidative stress response and life span in mammals. Nature (Lond.), *402:* 309–313, 1999.

95. Yang, F., von Knethen, A., and Brune, B. Modulation of nitric oxide-evoked apoptosis by the p53-downstream target p21(WAF1/CIP1). J. Leukocyte Biol., *68:* 916–922, 2000.

96. Bauer, P. M., Buga, G. M., and Ignarro, L. J. Role of p42/p44 mitogen-activated-protein kinase and p21waf1/cip1 in the regulation of vascular smooth muscle cell proliferation by nitric oxide. Proc. Natl. Acad. Sci. USA, *98:* 12802–12807, 2001.

97. Helt, C. E., Rancourt, R. C., Staversky, R. J., and O'Reilly, M. A. p53-dependent induction of p21(Cip1/WAF1/Sdi1) protects against oxygen-induced toxicity. Toxicol. Sci., *63:* 214–222, 2001.

98. Polyak, K., Waldman, T., He, T. C., Kinzler, K. W., and Vogelstein, B. Genetic determinants of p53-induced apoptosis and growth arrest. Genes Dev., *10:* 1945–1952, 1996.

99. Gorospe, M., Cirielli, C., Wang, X., Seth, P., Capogrossi, M., and Holbrook, N. P21 (WAF1/CIP1) protects against p53-mediated apoptosis of human melanoma cells. Oncogene, *14:* 929–935, 1997.

100. Kim, D. K., Cho, E. S., Lee, S. J., and Um, H. D. Constitutive hyperexpression of p21(WAF1) in human U266 myeloma cells blocks the lethal signaling induced by oxidative stress but not by Fas. Biochem. Biophys. Res. Commun., *289:* 34–38, 2001.

101. Yan, Q., and Sage, E. H. Transforming growth factor-$\beta$1 induces apoptotic cell death in cultured retinal endothelial cells but not pericytes: association with decreased expression of p21waf1/cip1. J. Cell. Biochem., *70:* 70–83, 1998.

102. Javelaud, D., Wietzerbin, J., Delattre, O., and Besancon, F. Induction of p21Waf1/Cip1 by TNF$\alpha$ requires NF-$\kappa$B activity and antagonizes apoptosis in Ewing tumor cells. Oncogene, *19:* 61–68, 2000.

103. Zhu, J., Jiang, J., Zhou, W., and Chen, X. The potential tumor suppressor p73 differentially regulates cellular p53 target genes. Cancer Res., *58:* 5061–5065, 1998.

104. Pennington, K. N., Taylor, J. A., Bren, G. D., and Paya, C. V. I$\kappa$B kinase-dependent chronic activation of NF-$\kappa$B is necessary for p21(WAF1/Cip1) inhibition of differentiation-induced apoptosis of monocytes. Mol. Cell. Biol., *21:* 1930–1941, 2001.

105. Donato, N. J., and Perez, M. Tumor necrosis factor-induced apoptosis stimulates p53 accumulation and p21WAF1 proteolysis in ME-180 cells. J. Biol. Chem., *273:* 5067–5072, 1998.

106. Glaser, T., Wagenknecht, B., and Weller, M. Identification of p21 as a target of cycloheximide-mediated facilitation of CD95-mediated apoptosis in human malignant glioma cells. Oncogene, *20:* 4757–4767, 2001.

107. Hobeika, A. C., Etienne, W., Torres, B. A., Johnson, H. M., and Subramaniam, P. S. IFN-γ induction of p21(WAF1) is required for cell cycle inhibition and suppression of apoptosis. J. Interferon Cytokine Res., *19:* 1351–1361, 1999.

108. Xaus, J., Cardo, M., Valledor, A. F., Soler, C., Lloberas, J., and Celada, A. Interferon γ induces the expression of p21waf-1 and arrests macrophage cell cycle, preventing induction of apoptosis. Immunity, *11:* 103–113, 1999.

109. Xaus, J., Comalada, M., Cardo, M., Valledor, A. F., and Celada, A. Decorin inhibits macrophage colony-stimulating factor proliferation of macrophages and enhances cell survival through induction of p27(Kip1) and p21(Waf1). Blood, *98:* 2124–2133, 2001.

110. Bellido, T., O'Brien, C. A., Roberson, P. K., and Manolagas, S. C. Transcriptional activation of the *p21(WAF1, CIP1, SDI1)* gene by interleukin-6 type cytokines. A prerequisite for their pro-differentiating and anti-apoptotic effects on human osteoblastic cells. J. Biol. Chem., *273:* 21137–21144, 1998.

111. Suzuki, T., Yokozaki, H., Kuniyasu, H., Hayashi, K., Naka, K., Ono, S., Ishikawa, T., Tahara, E., and Yasui, W. Effect of trichostatin A on cell growth and expression of cell cycle- and apoptosis-related molecules in human gastric and oral carcinoma cell lines. Int. J. Cancer, *88:* 992–997, 2000.

112. Chai, F., Evdokiou, A., Young, G. P., and Zalewski, P. D. Involvement of p21(Waf1/Cip1) and its cleavage by DEVD-caspase during apoptosis of colorectal cancer cells induced by butyrate. Carcinogenesis (Lond.), *21:* 7–14, 2000.

113. Mahyar-Roemer, M., and Roemer, K. p21 Waf1/Cip1 can protect human colon carcinoma cells against p53-dependent and p53-independent apoptosis induced by natural chemopreventive and therapeutic agents. Oncogene, *20:* 3387–3398, 2001.

114. Vrana, J. A., Decker, R. H., Johnson, C. R., Wang, Z., Jarvis, W. D., Richon, V. M., Ehinger, M., Fisher, P. B., and Grant, S. Induction of apoptosis in U937 human leukemia cells by suberoylanilide hydroxamic acid (SAHA) proceeds through pathways that are regulated by Bcl-2/Bcl-XL, c-Jun, and p21CIP1, but independent of p53. Oncogene, *18:* 7016–7025, 1999.

115. Burgess, A. J., Pavey, S., Warrener, R., Hunter, L. J., Piva, T. J., Musgrove, E. A., Saunders, N., Parsons, P. G., and Gabrielli, B. G. Up-regulation of p21(WAF1/CIP1) by histone deacetylase inhibitors reduces their cytotoxicity. Mol. Pharmacol., *60:* 828–837, 2001.

116. Gorospe, M., Wang, X., Guyton, K., and Holbrook, N. Protective role of p21 (WAF1/CIP1) against prostaglandin A2-mediated apoptosis of human colorectal carcinoma cells. Mol. Cell. Biol., *16:* 6654–6660, 1996.

117. Hitomi, M., Shu, J., Strom, D., Hiebert, S. W., Harter, M. L., and Stacey, D. W. Prostaglandin A2 blocks the activation of G1 phase cyclin-dependent kinase without altering mitogen-activated protein kinase stimulation. J. Biol. Chem., *271:* 9376–9383, 1996.

118. Gorospe, M., Shack, S., Guyton, K., Samid, D., and Holbrook, N. Up-regulation and functional role of p21 (WAF1/CIP1) during growth arrest of human breast carcinoma MCF-7 cells by phenylacetate. Cell Growth Differ., *7:* 1609–1615, 1996.

119. Ruan, S., Okcu, M. F., Ren, J. P., Chiao, P., Andreeff, M., Levin, V., and Zhang, W. Overexpressed WAF1/Cip1 renders glioblastoma cells resistant to chemotherapy agents 1,3-bis(2-chloroethyl)-1-nitrosourea and cisplatin. Cancer Res., *58:* 1538–1543, 1998.

120. Wang, Y., Blandino, G., and Givol, D. Induced p21waf expression in H1299 cell line promotes cell senescence and protects against cytotoxic effect of radiation and doxorubicin. Oncogene, *18:* 2643–2649, 1999.

121. Xu, G. W., Nutt, C. L., Zlatescu, M. C., Keeney, M., Chin-Yee, I., and Cairncross, J. G. Inactivation of p53 sensitizes U87MG glioma cells to 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res., *61:* 4155–4159, 2001.

122. Lin, H. M., Moon, B. K., Yu, F., and Kim, H. R. Galectin-3 mediates genistein-induced $G_2$/M arrest and inhibits apoptosis. Carcinogenesis (Lond.), *21:* 1941–1945, 2000.

123. Wang, Z., Van Tuyle, G., Conrad, D., Fisher, P. B., Dent, P., and Grant, S. Dysregulation of the cyclin-dependent kinase inhibitor p21WAF1/CIP1/MDA6 increases the susceptibility of human leukemia cells (U937) to 1-β-D-arabinofuranosylcytosine-mediated mitochondrial dysfunction and apoptosis. Cancer Res., *59:* 1259–1267, 1999.

124. Wang, Y. A., Elson, A., and Leder, P. Loss of p21 increases sensitivity to ionizing radiation and delays the onset of lymphoma in ATM-deficient mice. Proc. Natl. Acad. Sci. USA, *94:* 14590–14595, 1997.

125. Marches, R., Hsueh, R., and Uhr, J. W. Cancer dormancy and cell signaling: induction of p21(waf1) initiated by membrane IgM engagement increases survival of B lymphoma cells. Proc. Natl. Acad. Sci. USA, *96:* 8711–8715, 1999.

126. Wouters, B. G., Giaccia, A. J., Denko, N. C., and Brown, J. M. Loss of p21Waf1/Cip1 sensitizes tumors to radiation by an apoptosis-independent mechanism. Cancer Res., *57:* 4703–4706, 1997.

127. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer: correlation and relapse and survival with amplification of the HER-2/*neu* oncogene. Science (Wash. DC), *235:* 177–182, 1987.

128. Yu, D., Liu, B., Tan, M., Li, J., Wang, S. S., and Hung, M. C. Overexpression of c-erbB-2/neu in breast cancer cells confers increased resistance to Taxol via mdr-1-independent mechanisms. Oncogene, *13:* 1359–1365, 1996.

129. Horwitz, S. B. Mechanism of action of Taxol. Trends Pharmacol. Sci., *13:* 134–136, 1992.

130. Linke, S. P., Clarkin, K. C., Di Leonardo, A., Tsou, A., and Wahl, G. M. A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Genes Dev., *10:* 934–947, 1996.

131. Stewart, Z. A., Mays, D., and Pietenpol, J. A. Defective G1-S cell cycle checkpoint function sensitizes cells to microtubule inhibitor-induced apoptosis. Cancer Res., *59:* 3831–3837, 1999.

132. Li, W., Fan, J., Banerjee, D., and Bertino, J. R. Overexpression of p21(waf1) decreases G2-M arrest and apoptosis induced by paclitaxel in human sarcoma cells lacking both p53 and functional Rb protein. Mol. Pharmacol., *55:* 1088–1093, 1999.

133. Schmidt, M., Lu, Y., Liu, B., Fang, M., Mendelsohn, J., and Fan, Z. Differential modulation of paclitaxel-mediated apoptosis by p21Waf1 and p27Kip1. Oncogene, *19:* 2423–2429, 2000.

134. Zhou, B. P., Liao, Y., Xia, W., Spohn, B., Lee, M. H., and Hung, M. C. Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. Nat. Cell Biol., *3:* 245–252, 2001.

135. Asada, M., Yamada, T., Ichijo, H., Delia, D., Miyazono, K., Fukumuro, K., and Mizutani, S. Apoptosis inhibitory activity of cytoplasmic p21(Cip1/WAF1) in monocytic differentiation. EMBO J., *18:* 1223–1234, 1999.

136. Yu, D., Jing, T., Liu, B., Yao, J., Tan, M., McDonnell, T. J., and Hung, M. C. Overexpression of ErbB2 blocks Taxol-induced apoptosis by up-regulation of p21Cip1, which inhibits p34Cdc2 kinase. Mol. Cell, *2:* 581–591, 1998.

137. Li, Y., Dowbenko, D., and Lasky, L. A. AKT/PKB phosphorylation of p21Cip1/WAF1 enhances protein stability of p21Cip1/WAF1 and promotes cell survival. J. Biol. Chem., *277:* 11352–11361, 2002.

138. Lawlor, M. A., and Rotwein, P. Insulin-like growth factor-mediated muscle cell survival: central roles for Akt and cyclin-dependent kinase inhibitor p21. Mol. Cell. Biol., *20:* 8983–8995, 2000.

139. Wang, J., and Walsh, K. Resistance to apoptosis conferred by cdk inhibitors during myocyte differentiation. Science (Wash. DC), *273:* 359–361, 1996.

140. Fujio, Y., Guo, K., Mano, T., Mitsuuchi, Y., Testa, J. R., and Walsh, K. Cell cycle withdrawal promotes myogenic induction of Akt, a positive modulator of myocyte survival. Mol. Cell. Biol., *19:* 5073–5082, 1999.

141. Poluha, W., Poluha, D. K., Chang, B., Crosbie, N. E., Schonhoff, C. M., Kilpatrick, D. L., and Ross, A. H. The cyclin-dependent kinase inhibitor p21 (WAF1) is required for survival of differentiating neuroblastoma cells. Mol. Cell. Biol., *16:* 1335–1341, 1996.

142. Raj, K., Ogston, P., and Beard, P. Virus-mediated killing of cells that lack p53 activity. Nature (Lond.), *412:* 914–917, 2001.

143. Vogelstein, B., and Kinzler, K. W. Achilles' heel of cancer? Nature (Lond.), *412:* 865–866, 2001.

144. Schmidt, M., and Fan, Z. Protection against chemotherapy-induced cytotoxicity by cyclin-dependent kinase inhibitors (CKI) in CKI-responsive cells compared with CKI-unresponsive cells. Oncogene, *20:* 6164–6171, 2001.

145. Levkau, B., Koyama, H., Raines, E. W., Clurman, B. E., Herren, B., Orth, K., Roberts, J. M., and Ross, R. Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. Mol. Cell, *1:* 553–563, 1998.

146. Gervais, J. L., Seth, P., and Zhang, H. Cleavage of CDK inhibitor p21(Cip1/Waf1) by caspases is an early event during DNA damage-induced apoptosis. J. Biol. Chem., *273:* 19207–19212, 1998.

147. Jin, Y. H., Yoo, K. J., Lee, Y. H., and Lee, S. K. Caspase 3-mediated cleavage of p21WAF1/CIP1 associated with the cyclin A-cyclin-dependent kinase 2 complex is a prerequisite for apoptosis in SK-HEP-1 cells. J. Biol. Chem., *275:* 30256–30263, 2000.

148. Adachi, S., Ito, H., Tamamori-Adachi, M., Ono, Y., Nozato, T., Abe, S., Ikeda, M., Marumo, F., and Hiroe, M. Cyclin A/cdk2 activation is involved in hypoxia-induced apoptosis in cardiomyocytes. Circ. Res., *88:* 408–414, 2001.

149. Harvey, K. J., Lukovic, D., and Ucker, D. S. Caspase-dependent Cdk activity is a requisite effector of apoptotic death events. J. Cell Biol., *148:* 59–72, 2000.

150. de La Fuente, C., Santiago, F., Chong, S. Y., Deng, L., Mayhood, T., Fu, P., Stein, D., Denny, T., Coffman, F., Azimi, N., Mahieux, R., and Kashanchi, F. Overexpression of p21(waf1) in human T-cell lymphotropic virus type 1-infected cells and its association with cyclin A/cdk2. J. Virol., *74:* 7270–7283, 2000.

151. Xu, S. Q., and El-Deiry, W. S. p21(WAF1/CIP1) inhibits initiator caspase cleavage by TRAIL death receptor DR4. Biochem. Biophys. Res. Commun., *269:* 179–190, 2000.

152. Suzuki, A., Tsutomi, Y., Akahane, K., Araki, T., and Miura, M. Resistance to Fas-mediated apoptosis: activation of caspase 3 is regulated by cell cycle regulator p21WAF1 and IAP gene family ILP. Oncogene, *17:* 931–939, 1998.

153. Steinman, R. A., and Johnson, D. E. p21WAF1 prevents down-modulation of the apoptotic inhibitor protein c-IAP1 and inhibits leukemic apoptosis. Mol. Med., *6:* 736–749, 2000.

154. Suzuki, A., Tsutomi, Y., Miura, M., and Akahane, K. Caspase 3 inactivation to suppress Fas-mediated apoptosis: identification of binding domain with p21 and ILP and inactivation machinery by p21. Oncogene, *18:* 1239–1244, 1999.

155. Suzuki, A., Tsutomi, Y., Yamamoto, N., Shibutani, T., and Akahane, K. Mitochondrial regulation of cell death: mitochondria are essential for procaspase 3-p21 complex formation to resist Fas-mediated cell death. Mol. Cell. Biol., *19:* 3842–3847, 1999.

156. Fotedar, R., Brickner, H., Saadatmandi, N., Rousselle, T., Diederich, L., Munshi, A., Jung, B., Reed, J. C., and Fotedar, A. Effect of *p21waf1/cip1* transgene on radiation induced apoptosis in T cells. Oncogene, *18:* 3652–3658, 1999.

157. Shibata, M. A., Yoshidome, K., Shibata, E., Jorcyk, C. L., and Green, J. E. Suppression of mammary carcinoma growth *in vitro* and *in vivo* by inducible expression of the Cdk inhibitor p21. Cancer Gene Ther., *8:* 23–35, 2001.

158. Kondo, S., Barna, B. P., Kondo, Y., Tanaka, Y., Casey, G., Liu, J., Morimura, T., Kaakaji, R., Peterson, J. W., Werbel, B., and Barnett, G. H. WAF1/CIP1 increases the susceptibility of p53 non-functional malignant glioma cells to cisplatin-induced apoptosis. Oncogene, *13:* 1279–1285, 1996.

159. Lincet, H., Poulain, L., Remy, J. S., Deslandes, E., Duigou, F., Gauduchon, P., and Staedel, C. The p21(cip1/waf1) cyclin-dependent kinase inhibitor enhances the cytotoxic effect of cisplatin in human ovarian carcinoma cells. Cancer Lett., *161:* 17–26, 2000.

160. Kang, K. H., Kim, W. H., and Choi, K. H. p21 promotes ceramide-induced apoptosis and antagonizes the antideath effect of Bcl-2 in human hepatocarcinoma cells. Exp. Cell Res., *253:* 403–412, 1999.

161. Hsu, S. L., Chen, M. C., Chou, Y. H., Hwang, G. Y., and Yin, S. C. Induction of p21(CIP1/Waf1) and activation of p34(cdc2) involved in retinoic acid-induced apoptosis in human hepatoma Hep3B cells. Exp. Cell. Res., *248:* 87–96, 1999.

162. Li, Y., Upadhyay, S., Bhuiyan, M., and Sarkar, F. H. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. Oncogene, *18:* 3166–3172, 1999.

163. Tsao, Y. P., Huang, S. J., Chang, J. L., Hsieh, J. T., Pong, R. C., and Chen, S. L. Adenovirus-mediated *p21(WAF1/SDII/CIP1)* gene transfer induces apoptosis of human cervical cancer cell lines. J. Virol., *73:* 4983–4990, 1999.

164. Kadowaki, Y., Fujiwara, T., Fukazawa, T., Shao, J., Yasuda, T., Itoshima, T., Kagawa, S., Hudson, L. G., Roth, J. A., and Tanaka, N. Induction of differentiation-dependent apoptosis in human esophageal squamous cell carcinoma by adenovirus-mediated *p21sdi1* gene transfer. Clin. Cancer Res., *5:* 4233–4241, 1999.

165. Yang, Z. Y., Perkins, N. D., Ohno, T., Nabel, E. G., and Nabel, G. J. The p21 cyclin-dependent kinase inhibitor suppresses tumorigenicity *in vivo*. Nat. Med., *1:* 1052–1056, 1995.

166. Hingorani, R., Bi, B., Dao, T., Bae, Y., Matsuzawa, A., and Crispe, I. N. CD95/Fas signaling in T lymphocytes induces the cell cycle control protein p21cip-1/WAF-1, which promotes apoptosis. J. Immunol., *164:* 4032–4036, 2000.

167. Goke, R., Goke, A., Goke, B., El-Deiry, W. S., and Chen, Y. Pioglitazone inhibits growth of carcinoid cells and promotes TRAIL-induced apoptosis by induction of p21waf1/cip1. Digestion, *64:* 75–80, 2001.

168. Shin, B. A., Ahn, K. Y., Kook, H., Koh, J. T., Kang, I. C., Lee, H. C., and Kim, K. K. Overexpressed human RAD50 exhibits cell death in a p21(WAF1/CIP1)-dependent manner: its potential utility in local gene therapy of tumor. Cell Growth Differ., *12:* 243–254, 2001.

169. Chinery, R., Brockman, J., Peeler, M. O., Shyr, Y., Beauchamp, R. D., and Coffey, R. J. Antioxidants enhance the cytotoxicity of chemotherapeutic agents in colorectal cancer: a p53-independent induction of p21 (WAF1/CIP1) via C/EBP$\beta$. Nat. Med., *3:* 1233–1241, 1997.

170. Bach, S. P., Chinery, R., O'Dwyer, S. T., Potten, C. S., Coffey, R. J., and Watson, A. J. Pyrrolidine dithiocarbamate increases the therapeutic index of 5-fluorouracil in a mouse model. Gastroenterology, *118:* 81–89, 2000.

171. Ahmad, N., Adhami, V. M., Afaq, F., Feyes, D. K., and Mukhtar, H. Resveratrol causes WAF-1/p21-mediated $G_1$-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. Clin. Cancer Res., *7:* 1466–1473, 2001.

172. Evan, G. I., and Vousden, K. H. Proliferation, cell cycle and apoptosis in cancer. Nature (Lond.), *411:* 342–348, 2001.

173. Gartel, A. L., Serfas, M. S., and Tyner, A. L. p21-negative regulator of the cell cycle. Proc. Soc. Exp. Biol. Med., *213:* 138–149, 1996.