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Determine the Effect of p53 on Chemosensitivity

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Abstract

The p53 tumor suppressor protein plays a central role in mediating the cellular response to a variety of stresses. Activation of p53 signaling will trigger cell cycle arrest or apoptosis in normal cells, depending on such factors as cell type and genetic context. The ability of a cell to circumvent either of these p53-directed outcomes leads to inappropriate proliferation, thereby contributing to the development of cancer. As such, tumors frequently escape the apoptotic pathway in response to cell stress. DNA-damaging agents, however, achieve significant tumor cytotoxicity in spite of this hallmark characteristic. Tumors treated with DNA-damaging drugs often undergo alternate forms of cell death, such as senescence or mitotic catastrophe, in addition to apoptosis that may ultimately lead to regression. Although not a predictor of chemotherapy response in patients per se, p53 status in tumor-derived cells is frequently a determinant of the death pathway promoted by these agents. The cytotoxic effects of DNA-damaging agents can be readily appreciated using such tools as cell cycle analysis, phospho-H3^{Ser10} immunoblotting, and annexin V detection.

Keywords

p53; Tumor cells; DNA damage; Chemotherapy; Chemosensitivity; Drug response; Cell cycle; Apoptosis; Senescence; Mitotic catastrophe

1. Introduction

The p53 tumor suppressor protein is responsible for impeding the proliferation of cells in response to a variety of stresses, including genotoxic stress (1). In normal, healthy cells, p53 achieves this outcome by triggering either cell cycle arrest at the G1/S and G2/M checkpoints or apoptosis (2). Cells are thus able to prevent the propagation of potentially oncogenic mutations or other genetic aberrations. A testament to the critical role of p53 in protecting against such genomic instability is the observation that p53 is the most commonly mutated gene in human malignancies (2). Furthermore, p53-null mice develop tumors at an accelerated rate, often succumbing to disease by 6 months (3). Loss of p53 function constitutes a mechanism of checkpoint bypass and escape from apoptosis, the latter of which is a well-established hallmark of cancer (4).

Initial studies of cellular response to antineoplastic agents put forth that p53-directed apoptosis was the common underlying mechanism of chemotherapy and radiation response in tumors (5, 6). Indeed, certain tumors, particularly hematologic malignancies, undergo a robust, p53-dependent apoptotic response to extrinsic DNA damage (7). These findings further suggested that loss of p53 by mutation or deletion would render a given tumor resistant to treatment with genotoxic agents. Subsequent work in p53-null cells and animal

models, however, revealed that DNA-damaging treatments are able to achieve significant cytotoxicity in a p53-independent manner (8–10) (Fig. 1). Moreover, epithelial tumor lines and solid tumor models have been observed to respond to DNA damage despite not undergoing frank apoptosis (11). As the overwhelming majority of human malignancies are epithelial in origin, these alternate forms of DNA damage-induced tumor cell death are of particular interest in assessing tumor response to conventional cancer treatments, including topoisomerase inhibitors, cross-linking agents, and ionizing radiation.

Tumors in which the wild-type p53 gene remains expressed respond to DNA damage in a cell type- and genetic context-dependent manner, as discussed elsewhere (2). Under such circumstances, p53 and its downstream effectors may remain intact and responsive to DNA damage but have become insensitive to other oncogenic stresses (e.g., signaling stress) as a result of mutations in genes encoding upstream p53 activators (e.g., p14^{ARF}). Treatment of many epithelial neoplasms with DNA-damaging agents in cell culture, for example, will often provoke p53-dependent arrest at the G1/S and G2/M checkpoints that will prevent further progression through the cell cycle until the damage is repaired (12, 13). Removal of the offending compound will lead to eventual resolution of these checkpoints and resumption of the cell cycle (12). If DNA damage persists, perhaps by means of an effective dosing strategy, cells arrested in the cell cycle may ultimately senesce, as discussed below. Alternatively, tumors that arise from tissues known to be exquisitely sensitive to DNA damage, such as bone marrow or thymus, forego cell cycle arrest in favor of robust p53-dependent apoptosis (14, 15). In these tumors, loss of p53 has been associated with resistance to DNA-damaging therapies (5) (Fig. 1). The mechanistic basis of these divergent p53-dependent outcomes in response to DNA damage is a subject of continued investigation.

More recently, sustained p53 activation in the setting of DNA damage has also been shown to trigger accelerated cell senescence (16). Senescence refers to a permanent growth-arrested state in normal cells that have achieved their replicative lifespan, at which point telomere attrition has progressed so far as to expose chromosome ends (16, 17). Cells interpret the latter lesion as a double-stranded DNA break, driving sustained p53 signaling that yields viable, metabolically active but growth-arrested cells with readily discernible characteristics (16). These features include a flattened morphology, densely vacuolated cytoplasm, and strong staining for senescence-associated (SA) β -galactosidase (Fig. 2) (16). Although cell senescence does not eradicate tumor cell populations in culture, animal models have suggested that senescent tumors are cleared by the innate immune system in vivo (18).

Despite the high frequency of p53 mutations in cancer, the absence of p53-mediated outcomes does not correlate with a failure of solid tumors to respond to DNA-damaging treatments (19). Cells that have lost p53 are deficient in G1/S arrest but remain capable of p53-independent arrest at the G2/M checkpoint, albeit transiently (20). Under conditions of sustained DNA damage, these cells escape G2 arrest with unrepaired DNA (Fig. 3). The resulting abnormal mitosis gives way to chromosome mis-segregation, cell fusion, micronuclei formation, and multinucleated cells in a process termed “mitotic catastrophe” (21, 22). Mitotic catastrophe eventually triggers cell death that may occasionally share some

features of apoptosis (e.g., caspase activation) but is nevertheless distinct in that it is preceded by the above-captioned atypia (23).

The effect of p53 status on DNA damage-directed cell fate can be readily assessed by way of propidium iodide staining and flow cytometry. This technique is complemented by immunoblotting for Serine 10-phosphorylated histone H3, an epigenetic alteration that accompanies chromosome condensation and segregation during mitosis (24) (Fig. 3). Apoptotic cell death can also be assayed by way of flow cytometry-directed detection of immunolabeled annexin V. Annexin V binds with high affinity to phosphatidylserine, which is known to be exposed on the outer leaflet of the plasma membrane of cells undergoing apoptosis (25) (Fig. 4).

2. Materials

2.1. Cell Culture, Drug Treatment, and Harvest

1. Dulbecco's modified Eagle's medium (DMEM) with High Glucose, supplemented with 10% fetal bovine serum (FBS).
2. 0.05% Trypsin–ethylenediaminetetraacetic acid (EDTA).
3. Doxorubicin HCl (Sigma-Aldrich, St. Louis, MO), dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO). 1 mg/mL stocks should be prepared and aliquoted after filtration through a 0.2- μ m filter under a laminar flow hood and maintained at -20°C thereafter. Working dilutions can also be maintained at -20°C after 1:10 dilution in sterile water.

2.2. Propidium Iodide Staining for FACS Analysis

1. DMEM with High Glucose, supplemented with 10% FBS.
2. 0.05% Trypsin–EDTA.
3. 70% Ethanol (EtOH).
4. 100 $\times$ Stock propidium iodide (Sigma-Aldrich, St. Louis, MO) prepared by dissolving available powder in PBS. Stock is stored in the dark at 4°C .
5. Ribonuclease A (RNase A) powder, prepared from bovine pancreas (Sigma-Aldrich, St. Louis, MO). Powder is stored as-is at -20°C .
6. Conical-bottom 15 mL Falcon tubes, 17 $\times$ 120 mm style (BD Falcon, Franklin Lanes, NJ).
7. Round-bottom 12 $\times$ 75 mm Falcon tubes, polystyrene (BD Falcon, Franklin Lanes, NJ).

2.3. SDS-PAGE, Electrophoretic Transfer, and Immunoblotting for Serine 10-Phosphorylated Histone H3

1. Tetramethylethylenediamine (TEMED) (Bio-Rad Laboratories, Rockaway, NJ).

2. 10% Ammonium persulfate (Sigma-Aldrich, St. Louis, MO) dissolved in distilled water. Store at 4°C for up to 1 month.
3. 30% Acrylamide/Bis solution, 37.5:1 (2–8°C) (Sigma-Aldrich, St. Louis, MO).
4. 10% Sodium-dodecyl sulfate (SDS) (Invitrogen, Carlsbad, CA) in distilled water.
5. 12% Resolving gel mix: Add 3 mL 10% SDS to 75 mL 1.5 M *tris*(hydroxymethyl)aminomethane (TRIS) (Sigma-Aldrich, St. Louis, MO), diluted in distilled water, and pH adjusted to 8.8 with HCl. Bring final volume to 200 mL with distilled water. Store at 4°C.
6. 5% Stacking gel mix: Add 3 mL 10% SDS to 75 mL 0.5 M TRIS (Sigma-Aldrich, St. Louis, MO), diluted in distilled water, and pH adjusted to 6.8 with HCl. Bring final volume to 250 mL with distilled water. Store at 4°C.
7. 1× SDS-PAGE running (Tris/Glycine/SDS) buffer (Bio-Rad Laboratories, Rockaway, NJ).
8. 0.1% Polyethylene glycol sorbitan monolaurate (TWEEN®20) dissolved in 1× PBS (Sigma-Aldrich, St. Louis, MO).
9. 3MM blotting paper (Whatman, Piscataway, NJ).
10. Nitrocellulose membranes (Bio-Rad Laboratories, Rockaway, NJ).
11. Gel transfer buffer. Prepare 1 L using 100 mL 10× Tris/Glycine buffer (Bio-Rad Laboratories, Rockaway, NJ), 200 mL 195-proof EtOH, and 700 mL distilled water.
12. Blocking solution: 2.5% nonfat dry milk (LabScientific, Livingston, NJ) dissolved in 1× PBS/0.1% TWEEN®20.
13. Rabbit polyclonal Anti-Phospho-Histone H3^{Ser10} antibody (Millipore, Billerica, MA), diluted 1:10,000 in blocking solution.
14. Stock lysis buffer reagents:

Lysis buffer

 - (a) 10% Triton-X-100 (FisherScientific, Fair Lawn, NJ).
 - (b) 1 M HEPES pH 7.5 (FisherScientific, Fair Lawn, NJ).
 - (c) 5 M NaCl (FisherScientific, Fair Lawn, NJ).
 - (d) 1 M MgCl₂ (FisherScientific, Fair Lawn, NJ).

Stock Triton-X-100-base may be prepared with final concentrations of 1% Triton-X-100, 50 mM HEPES pH 7.5, 50 mM NaCl, 1 mM MgCl₂ in an appropriate volume of distilled water. The following inhibitors are added to this buffer base immediately prior to use.:

Phosphatase inhibitors

- (a) 0.1 M Sodium orthovanadate (Sigma-Aldrich, St. Louis, MO). Store stock at -20°C . Use 10 mL per mL of lysis buffer.
- (b) 0.5 M Sodium fluoride (Sigma-Aldrich, St. Louis, MO). Store stock at -20°C . Use 2 mL per mL of lysis buffer.
- (c) 1.5 M 4-Nitrophenyl phosphate disodium salt hexahydrate (Sigma-Aldrich, St. Louis, MO). Store stock at -20°C . Use 1 μL per mL of lysis buffer.

Protease inhibitors

- (a) 50 mM Phenylmethylsulfonyl fluoride (Sigma-Aldrich, St. Louis, MO). Store stock at -20°C . Use 20 μL per mL of lysis buffer.
 - (b) Aprotinin, from bovine pancreas (Sigma-Aldrich, St. Louis, MO). Store at 4°C . Use 30 μL per mL of lysis buffer.
 - (c) 2 mg/mL leupeptin (Sigma-Aldrich, St. Louis, MO). Store stock at -20°C . Use 1 μL per mL of lysis buffer.
15. $3\times$ Protein sample buffer, prepared with 3 mL glycerol, 1.5 mL β -mercaptoethanol, 0.9 g SDS, 3.75 mL 0.5 M TRIS PO_4 pH 6.8 (all available through FisherScientific, Fair Lawn, NJ), adjusted to 10 mL with water. 1.0 mL Aliquots should be stored at -20°C .
 16. 1.7 mL Microcentrifuge tubes (VWR Scientific, Buffalo Grove, IL).
 17. Anti-actin rabbit polyclonal antibody (Sigma-Aldrich, St. Louis, MO), diluted 1:1,000 in $1\times\text{PBS}/0.1\%$ TWEEN[®]20.
 18. Broad range protein standards (Fermentas, Glen Burnie, MD).
 19. HRP-conjugated goat anti-rabbit secondary antibody (Millipore, Billerica, MA), diluted 1:1,000 in blocking solution.
 20. Enhanced chemiluminescence (ECL) substrates for HRP detection (Thermo Scientific, Rockford, IL).
 21. Autoradiography film and cassettes (MidSci, St. Louis, MO).

2.4. Annexin V Detection by Flow Cytometry

1. FITC-conjugated anti-Annexin V antibody (BD Pharmingen, San Diego, CA).
2. Annexin V binding buffer (AVBB) stock reagents:
 - (a) 1 M HEPES pH 7.5 (FisherScientific, Fair Lawn, NJ).
 - (b) 5 M NaCl (FisherScientific, Fair Lawn, NJ).
 - (c) 1 M MgCl_2 (FisherScientific, Fair Lawn, NJ).
 - (d) 1 M CaCl_2 (FisherScientific, Fair Lawn, NJ).

Prepare AVBB with final concentrations of reagents as follows: 10 mM HEPES pH 7.5, 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, and 1.8 mM CaCl₂. AVBB should be stored at 4°C for up to 1 month.

2.5. Equipment

1. Laminar flow hood for sterile cell culture work.
2. CO₂ incubator, 37°C.
3. Refrigerator, 4°C.
4. Freezers, -20°C and -80°C.
5. Microcentrifuge.
6. Low speed centrifuge with swinging bucket rotor capable of accommodating 15 mL tubes (see Subheading 2.2, above).
7. FACS brand flow cytometer (BD BioSciences, San Jose, CA).
8. Appropriate apparatuses for SDS-acrylamide gel casting, SDS-PAGE, gel transfer, and subsequent immunoblotting (available through Bio-Rad Laboratories, Rockaway, NJ).
9. Dark room with film developer capable of processing autoradiographs.

3. Methods

3.1. Staining with Propidium Iodide for Cell Cycle Analysis

p53 and Cell Cycle Effects after DNA Damage Chapter 4, “Determine the effect of p53 on cell cycle,” for the relevant protocol.

3.2. Detection of Serine 10-Phosphorylated Histone H3 by Immunoblotting

It is recommended that SDS-PAGE gels be cast ahead of time. Gels may be stored at 4°C for up to 5 days:

12.0% Resolving gel: Combine 5.9 mL 12% gel mix, 4.0 mL 30% Acrylamide/Bis solution, 37.5:1, 100 µL 10% APS, and 10 µL TEMED. Mix thoroughly and add 7.5 mL to an 8 cm × 10 cm × 1.5 mm gel cassette. Overlay with 70% EtOH. Incubate 15 min at room temperature. Dispose of ethanol overlay and rinse gently five to six times with distilled water.

5.0% Stacking gel: Combine 8.3 mL 5.0% gel mix, 1.7 mL, 4.0 mL 30% Acrylamide/Bis solution, 37.5:1, 100 µL 10% APS, and 10 µL TEMED. Mix thoroughly and add a volume sufficient to fill casting apparatus. Insert a 10- or 15-well comb (depending on the number of samples), taking care not to introduce air bubbles. Incubate 15 min at room temperature.

1. Cells are harvested by trypsinization at relevant points in time following treatment (e.g., every 24 h). Cells should be spun down at 2,000 g × 5 min at 4°C, rinsed once with 1× PBS, and spun again. Pellets may be frozen for lysis at a later time,

provided that all supernatants are aspirated and pellets are kept on dry ice for 5–10 min prior to storage at -80°C .

2. Cell pellets are lysed in an appropriate amount of Triton-X-100-based lysis buffer, prepared with the addition of protease and phosphatase inhibitors as detailed in Subheading 2. Cells should be lysed on ice for 10–15 min.
3. Whole cell lysates (WCLs) are spun at $16,000\text{ g} \times 5\text{ min}$ at 4°C . The supernatant is transferred to a new 1.7 mL microcentrifuge tube. The pellet is discarded.
4. The protein content of each WCL is determined by Bradford assay.
5. Samples are prepared using a sufficient volume of WCL for 50 μg of protein in a 1.7 mL microcentrifuge tube. Volume across samples should be equalized using Triton-X-100 lysis buffer, as prepared above. Add an appropriate volume of 3 $\times$ protein sample buffer to each sample.
6. Samples are vortexed, spun down, and incubated at 95°C for 3 min to denature proteins. To avoid having tubes pop open during this incubation, a syringe needle may be used to produce a hole in the lid of each tube.
7. Samples are again vortexed and spun down after incubation.
8. Samples are loaded into the wells of a pre-cast 12.0% resolving/5.0% stacking gel. 10 μL Protein standards in an appropriate amount of 3 $\times$ protein sample buffer should be loaded into one lane at either end of the gel.
9. Samples are run at 130 V for approximately 1.5 h, until sample buffer has run out of the gel and into the running buffer.
10. After electrophoresis, the gel is transferred to a nitrocellulose membrane at 110 V for 60 min. Gel transfer should be carried out at 4°C .
11. The nitrocellulose membrane is cut horizontally at the $\sim 35\text{ kDa}$ marker. The top half of the membrane can be used for actin immunodetection ($\sim 43\text{ kDa}$). The bottom half is to be used for phospho- H3^{Ser10} immunodetection ($\sim 17\text{ kDa}$).
12. The nitrocellulose membrane sections are blocked in blocking solution for 1 h. The upper half is incubated in an appropriate volume and dilution of anti-actin antibody, the lower half in anti-phospho- H3^{Ser10} , overnight with gentle agitation.
13. The membrane sections are washed three times, 10 min each, in blocking solution.
14. The membrane sections are incubated in an appropriate volume and dilution of HRP-conjugated anti-rabbit secondary for 1 h at room temperature with gentle agitation.
15. The membrane sections are washed four times, 10 min each, in blocking solution. Two final washes in 1 $\times$ PBS/0.1% TWEEN[®]20, 10 min each, are performed to wash out any residual milk prior to developing.

16. The membranes are coated in ECL reagent for 1 min. The ECL is blotted off on a paper towel. The membranes are wrapped in transparent film and fixed to the inside of an auto-radiography cassette with clear tape.
17. In a dark room, membranes are exposed against autoradiography film for a range of different exposure lengths (e.g., 5, 10, 30, and 60 s). Films are developed and labeled appropriately.

3.3. Assessment of Apoptosis by Annexin V Staining and Detection by Flow Cytometry

1. Unlike PI staining for flow cytometry analysis of DNA content, cells harvested for Annexin V staining are not fixed in EtOH and cannot be stored at -20°C for later analysis. All samples must be harvested and analyzed at the same time. For assessment of up to 72 h of treatment, for example, cells should be split evenly 4 days prior to analysis and treated on days 3, 2, and 1 prior to harvest. An untreated plate of cells may be harvested on the day of analysis as a control sample.
2. Cells are harvested by trypsinization. Cell culture medium should be retained in a 15 mL conical-bottom Falcon tube, as this is likely to contain dead cells that will be relevant for the analysis. This medium can be used to neutralize the trypsinization reaction and collect adherent cells into the same tube. Add 2–3 mL of $1\times$ PBS and collect into relevant tube of cells to ensure maximum recovery.
3. Cell suspensions are spun at $800\text{ g} \times 5\text{ min}$ at room temperature.
4. Cell supernatant is aspirated. All visible traces of trypsin should be removed, as residual enzyme may cleave exposed annexin V.
5. The FITC-conjugated annexin V antibody is diluted 1:250 in AVBB.
6. Cells are resuspended in 500 μL to 1.0 mL of FITC-annexin V dilution and transferred to round-bottom $12 \times 75\text{ mm}$ Falcon tubes.
7. Cells are kept in the dark at 4°C for 30 min. Cells are resuspended every 5–10 min to ensure adequate antibody binding.
8. Samples are analyzed for annexin V expression on a BD BioSciences FACScalibur flow cytometer. The FL1 laser is used to detect the FITC-annexin V antibody. CellQuest software is used to generate both acquisition and analysis plots of 2,000 cells. The percent of cells that are annexin V positive is used as a surrogate measure for cells that have undergone apoptosis.

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References

1. Stewart ZA, Pietenpol JA. p53 Signaling and cell cycle checkpoints. *Chem Res Toxicol*. 2001; 14:243–263. [PubMed: 11258974]

2. Vousden KH, Lu X. Live or let die: the cell's response to p53. *Nat Rev Cancer*. 2002; 2:594–604. [PubMed: 12154352]
3. Sansom OJ, Clarke AR. P53 null mice: damaging the hypothesis? *Mutat Res*. 2000; 452:149–162. [PubMed: 11024475]
4. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000; 100:57–70. [PubMed: 10647931]
5. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell*. 1993; 74:957–967. [PubMed: 8402885]
6. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature*. 1993; 362:847–849. [PubMed: 8479522]
7. Gudkov AV, Komarova EA. The role of p53 in determining sensitivity to radiotherapy. *Nat Rev Cancer*. 2003; 3:117–129. [PubMed: 12563311]
8. Merritt AJ, Allen TD, Potten CS, Hickman JA. Apoptosis in small intestinal epithelial from p53-null mice: evidence for a delayed, p53-independent G2/M-associated cell death after gamma-irradiation. *Oncogene*. 1997; 14:2759–2766. [PubMed: 9190891]
9. Han JW, Dionne CA, Kederasha NL, Goldmacher VS. p53 status affects the rate of the onset but not the overall extent of doxorubicin-induced cell death in rat-1 fibroblasts constitutively expressing c-Myc. *Cancer Res*. 1997; 57:176–182. [PubMed: 8988061]
10. Tannock IF, Lee C. Evidence against apoptosis as a major mechanism for reproductive cell death following treatment of cell lines with anti-cancer drugs. *Br J Cancer*. 2001; 84:100–105. [PubMed: 11139321]
11. Kemp CJ, Sun S, Gurley KE. p53 induction and apoptosis in response to radio- and chemotherapy in vivo is tumor-type-dependent. *Cancer Res*. 2001; 61:327–332. [PubMed: 11196181]
12. Agarwal ML, Agarwal A, Taylor WR, Stark GR. p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. *Proc Natl Acad Sci USA*. 1995; 92:8493–8497. [PubMed: 7667317]
13. Platt PM, Tang LJ, Scatena CD, Szak ST, Pietenpol JA. p53 regulation of G(2) checkpoint is retinoblastoma protein dependent. *Mol Cell Biol*. 2000; 20:4210–4223. [PubMed: 10825186]
14. Meulmeester E, Jochemsen AG. p53: a guide to apoptosis. *Curr Cancer Drug Targets*. 2008; 8:87–97. [PubMed: 18336191]
15. Schmitt CA, Rosenthal CT, Lowe SW. Genetic analysis of chemoresistance in primary murine lymphomas. *Nat Med*. 2000; 6:1029–1035. [PubMed: 10973324]
16. Gewirtz DA, Holt SE, Elmore LW. Accelerated senescence: an emerging role in tumor cell response to chemotherapy and radiation. *Biochem Pharmacol*. 2008; 76:947–957. [PubMed: 18657518]
17. Dimri GP. What has senescence got to do with cancer? *Cancer Cell*. 2005; 7:505–512. [PubMed: 15950900]
18. Xue W, Zender L, Miething C, Dickins RA, Hernandez E, Krizhanovsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature*. 2007; 445:656–660. [PubMed: 17251933]
19. Brown JM, Wilson G. Apoptosis genes and resistance to cancer therapy: what does the experimental and clinical data tell us? *Cancer Biol Ther*. 2003; 2:477–490. [PubMed: 14614312]
20. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science*. 1998; 282:1497–1501. [PubMed: 9822382]
21. Castedo M, Perfettini JL, Roumier T, Andreau K, Medema R, Kroemer G. Cell death by mitotic catastrophe: a molecular definition. *Oncogene*. 2004; 23:2825–2837. [PubMed: 15077146]
22. Mansilla S, Bataller M, Portugal J. Mitotic catastrophe as a consequence of chemotherapy. *Anticancer Agents Med Chem*. 2006; 6:589–602. [PubMed: 17100562]
23. Mansilla S, Priebe W, Portugal J. Mitotic catastrophe results in cell death by caspase-dependent and caspase-independent mechanisms. *Cell Cycle*. 2006; 5:53–60. [PubMed: 16319532]
24. Nowak SJ, Corces VG. Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. *Trends Genet*. 2004; 20:214–220. [PubMed: 15041176]

25. Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood*. 1994; 84:1415–1420. [PubMed: 8068938]
26. DeWeese TL, Walsh JC, Dillehay LE, Kessis TD, Hedrick L, Cho KR, Nelson WG. Human papillomavirus E6 and E7 oncoproteins alter cell cycle progression but not radio-sensitivity of carcinoma cells treated with low-dose-rate radiation. *Int J Radiat Oncol Biol Phys*. 1997; 37:145–154. [PubMed: 9054890]
27. Portugal J, Bataller M, Mansilla S. Cell death pathways in response to antitumor therapy. *Tumori*. 2009; 95:409–421. [PubMed: 19856649]
28. Bunz F, Hwang PM, Torrance C, Waldman T, Zhang Y, Dillehay L, Williams J, Lengauer C, Kinzler KW, Vogelstein B. Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *J Clin Invest*. 1999; 104:263–269. [PubMed: 10430607]

Fig. 1.

DNA damage-induced apoptosis in human colorectal carcinoma cell line RKO and derivative line RKO E6, in which stable expression of the HPV E6 protein has been shown to promote the degradation of p53 (26). RKO cells respond to sustained DNA damage by undergoing p53-dependent apoptosis, revealed by an increase in the hypodiploid population of cells after treatment by PI staining and flow cytometry (**a**, *first column*). The same assay reveals RKO E6 cells, on the other hand, to be resistant to DNA damage-induced apoptosis (**a**, *second column*). Apoptosis in RKO cells can be quantified by way of annexin V immunolabeling and detection by flow cytometry, as shown in (**b**).

Fig. 2.

Accelerated senescence in response to DNA damage occurs in a p53-dependent manner. U2OS clone 1 cells, which overexpress a control shRNA, accumulate SA-β-gal and acquire the typical flattened morphology of senescent cells after 6 days of doxorubicin treatment. Conversely, U2OS clone 7 cells, which stably express shRNA to p53, demonstrate blebbing of their plasma membranes, a characteristic of p53-independent, apoptosis-like cell death (27).

Fig. 3.

Response to sustained DNA damage in paired HCT116 cell lines. HCT116 is a readily available, wild-type p53-expressing colorectal carcinoma cell line that has been subject to homologous recombination so as to produce an isogenic p53^{-/-} derivative (28). The parental line demonstrates sustained cell cycle arrest with decreased phospho-H3^{Ser10} detection. The p53^{-/-} line, however, achieves only transient cell cycle arrest at the G2/M checkpoint with eventual reentry into mitosis, evidenced by a detectable rise in phospho-H3^{Ser10} by immunoblotting. This rise in phospho-H3^{Ser10} accompanies the observed decay in the G2/M peak and an increase in the sub-G1 population of cells, indicative of mitotic catastrophe. At 72 h, phospho-H3^{Ser10} levels are seen to drop again as cells die. A sample of cells treated with 0.5 µg/mL nocodazole is a useful positive control (lanes labeled “NZ”). This agent arrests cells in prometaphase owing to its interference with the polymerization of microtubules.

Fig. 4. p53 status as a determinant of cell outcome in response to DNA damage. In tumors harboring wild-type p53, exposure to DNA damage results either p53-dependent apoptosis or cell cycle arrest at the G1/S and G2/M checkpoints. If the latter outcome persists, these cells may senesce. Senescent cells *in vivo* have been shown to be cleared by the innate immune system, ultimately leading to tumor regression (18). Tumors that have lost p53, on the other hand, will respond to DNA damage by transiently arresting at the G2/M checkpoint but eventually re-entering mitosis. The ensuing process, often referred to as mitotic catastrophe (21), results in a p53-independent form of cell death that follows chromosome mis-segregation, cell fusion, micronuclei formation, and the presence of multinucleated cells.