Doxorubicin is one of the most effective molecules used in the treatment of various tumors. Contradictory reports often open windows to understand the role of p53 tumor suppressor in doxorubicin-mediated cell death. In this report, we provide evidences that doxorubicin induced more cell death in p53-negative tumor cells. Several cells, having p53 basal expression, showed increase in p53 DNA binding upon doxorubicin treatment. Doxorubicin induced cell death in p53-positive cells through expression of p53-dependent genes and activation of caspases and caspase-mediated cleavage of cellular proteins. Surprisingly, in p53-negative cells, doxorubicin-mediated cell death was more aggressive (faster and intense). Doxorubicin increased the amount of Fas ligand (FasL) by enhancing activator protein (AP) 1 DNA binding in both p53-positive and p53-negative cells, but the basal expression was higher in p53-negative cells. Anti-FasL antibody considerably protected doxorubicin-mediated cell death of cells. Activation of caspases was faster in p53-negative cells upon doxorubicin treatment. In contrast, the basal expression of Ras oncoprotein was higher in p53-positive cells, which upon doxorubicin treatment. In contrast, the basal expression of Ras oncoprotein was higher in p53-positive cells, which upon doxorubicin treatment. In contrast, the basal expression of Ras oncoprotein was higher in p53-positive cells, which upon doxorubicin treatment. In contrast, the basal expression of Ras oncoprotein was higher in p53-positive cells, which upon doxorubicin treatment. In contrast, the basal expression of Ras oncoprotein was higher in p53-positive cells, which upon doxorubicin treatment.
**p53-negative Cells Are More Sensitive to Doxorubicin**

In this report, we have found that doxorubicin-mediated cell death is slow and less potent in p53-positive cells. Breast tumor cell line MCF-7 has basal expression of p53, whereas other breast cell lines such as SKBr3 and MDA-MB-231 have mutated or no p53 expression. HCT116 cells are knocked out of p53 by homologous recombination and designated as (HCT116 (p53⁻/⁻)), and non-transfected cells (HCT116 (Wild)) are used for this study. We have provided the evidences for the first time that p53-positive cells have high basal K-ras, but low Fas expression, which might dictate p53-positive cells for slow and less potent cell death mediated by doxorubicin than p53 mutant or null cells, although both types of cells showed equal expression of Fasl upon treatment of doxorubicin. This study will help understand how p53, although it is a well known tumor suppressor, puts the brake via the Fas-Ras pathway on doxorubicin-mediated cell death, which might help target the specific molecules to regulate sensitization of p53-positive cells for aggressive cell death.

**EXPERIMENTAL PROCEDURES**

**Materials**—Doxorubicin, EDTA, EGTA, PMSF, propidium iodide, Ac-DVED-pNA, Ac-ITED-pNA, 3-(4,5-dimethylthiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT), glycogen—DNA—GFP, PARP, K-ras, Fas, FasL, IL-8, goat anti-rabbit IgG conjugated with HRP, and gel shift antibodies against p53, p21, PARP, JNK1, Fas, FasL, K-ras, and THP1 cells were obtained from Invitrogen. DAPI and goat anti-rabbit IgG conjugated with the LIVE/DEAD assay (27) (Molecular Probes, Eugene, OR). Briefly, after different treatments, 1 × 10⁵ cells/well of a 96-well plate were densely plated for the indicated concentrations and times, and thereafter, 25 μl of MTT solution (5 mg/ml in PBS) was added. After a 2-h incubation, 100 μl of 10% SDS in 50% dimethylformamide was added. After an overnight incubation at 37 °C, absorbance was monitored with a luminometer, and values were calculated as -fold of activation over vector-transfected value.

**Cytotoxicity Assay**—The cytotoxicity was measured by MTT assay (15). The amount of Ras was examined by the immunocytochemical method as described (11).

**Immunocytochemistry**—The amount of Ras was examined by the immunocytochemical method as described (11). Briefly, cells were cultured on chamber slides, washed after different treatments, air-dried, fixed with 3.5% formaldehyde, and permeabilized with 0.5% Triton X-100. Slides were blocked by 5% goat serum and incubated with anti-K-ras Ab for 8 h followed by incubation with goat anti-rabbit IgG-Alexa Fluor 594 for 1 h. Slides were mounted with DAPI and analyzed under a fluorescence microscope (20).

**Determination of DNA Fragmentation**

DNA was extracted following the method described earlier (20). Extracted DNA (2.0 μg) was analyzed by electrophoresis on a 2% agarose gel. DNA fragments were visualized with ethidium bromide under UV light.

**Determination of Nuclear Fragmentation**

The cytotoxicity was determined by the LIVE/DEAD assay (27). Briefly, after different treatments, 1 × 10⁵ cells were stained with the LIVE/DEAD cell assay reagent. Red (as dead) and blue (as live) cells were analyzed under a fluorescence microscope (Labophot-2, Nikon, Tokyo, Japan).

**Caspase 3 and 8 Activities Assay**—To evaluate caspase 3 and 8 activities, cell lysates were prepared after their respective treatments. 50 μg of the cell lysate proteins was incubated with 200 μm caspase 3 substrate (Ac-DVED-pNA) or caspase 8 substrate (Ac-ITED-pNA) in 100 μl of reaction buffer (1%...
p53-negative Cells Are More Sensitive to Doxorubicin

Nonidet P-40, 20 μM Tris-HCl, pH 7.5, 137 mM NaCl, and 10% glycerol) and incubated for 2 h at 4°C. The JNK complex from whole-cell extract (300 μg) was precipitated with anti-JNK1 antibody (1:1000 dilution) and the number of JNK1 was quantified by chemiluminescence (Amersham Biosciences).

In Vitro JNK Assay

In this study, the effects of doxorubicin in p53-positive and p53-negative cells were studied. Doxorubicin was used as a solvent, dimethyl sulfoxide (DMSO) at 10 mM concentration. Cells were treated with 1 μM doxorubicin for different times, and inhibition of cell viability was calculated from the MTT assay.

Results

Different concentrations and duration of exposure of the chemicals increased cell death almost 15–25% at any time of treatment compared with MCF-7 cells. Doxorubicin was used as a solvent, dimethyl sulfoxide (DMSO) at 10 mM concentration. Cells were treated with 1 μM doxorubicin for different times, and inhibition of cell viability was calculated from the MTT assay.

FIGURE 1. Effect of doxorubicin on induction of cell death in U-937, HeLa, THP1, SKBr3, and MCF-7 cells. MCF-7 cells were treated with 1 μM doxorubicin for different times, and inhibition of cell viability was calculated from the MTT assay. B, U-937, THP1, MCF-7, HeLa, and SKBr3 cells were treated with 1 μM doxorubicin for 72 h. Cells were fixed, stained with propidium iodide, and viewed under a fluorescence microscope. C, the percentage of apoptotic cells is indicated in the figure. MCF-7 and HeLa cells were treated with doxorubicin for different times, and inhibition of cell viability was calculated from the MTT assay. D, U-937, THP1, MCF-7, HeLa, and SKBr3 cells were treated with doxorubicin for different times, and inhibition of cell viability was calculated from the MTT assay.

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p53-negative Cells Are More Sensitive to Doxorubicin

FIGURE 2. Effect of doxorubicin on induction of p53 and cell death in p53-positive and -negative cells. A, MCF-7 and SKBr3 cells were treated with different concentrations of doxorubicin for 24 h, and nuclear extracts were assayed for p53 DNA binding by gel shift assay. B, HCT116 cells (HCT116 (Wild)) and p53 knock-out cells (HCT116 (p53−/−)) were treated with 1 μM doxorubicin for different times, and nuclear extracts were assayed for p53 DNA binding by gel shift assay. Lower panels, whole-cell extracts were prepared, and 8 μg of NE proteins was used to detect p53 and NF-κB by gel shift assay. C, inhibition of cell viability was detected by MTT assay.

DEAD assay (Fig. 1C). At 24 h of doxorubicin treatment, most no DNA fragmentation was observed in MCF-7 cells, but HeLa cells showed sufficient DNA fragmentation (supplemental Fig. 1D). Partial PARP cleavage was observed in MCF-7 cells upon doxorubicin treatment, whereas HCT116 cells, complete PARP cleavage was observed (supplemental Fig. 1D). Phase contrast microscope views for the doxorubicin-treated cells also suggested more cell death in HeLa cells than in MCF-7 cells (supplemental Fig. 1B). These data suggest that doxorubicin-mediated cell death in HCT116 (Wild) cells is more than 20–25% more cell death than in HCT116 (p53−/−) cells at any point of doxorubicin treatment (supplemental Fig. 2B).

Doxorubicin Increases AP-1 DNA Binding, JNK Activation, FasL Expression, and Caspase Activation in Both p53-positive and p53-negative Cells—To understand the differential potency of doxorubicin-mediated cell death in p53-positive and -negative cells, we have measured the amount of FasL, which is transcriptionally regulated by AP-1 (18, 19), upon treatment of doxorubicin. Doxorubicin increased AP-1 DNA binding with increasing time of treatment as shown from the nuclear extracts by gel shift assay in both HCT116 (Wild) and HCT116 (p53−/−) cells (Fig. 3A, lower panel). It showed biphasic activation of NF-κB DNA binding in both types of cells (Fig. 3A, upper panel), which is supported by our previous observation (17). Doxorubicin almost equally induced JNK activation in both types of cells as shown by detecting the amount of phospho-JNK by Western blot (supplemental Fig. 3A) or by in vitro JNK assay using GST-Jun as substrate protein (Fig. 3B). The amount of FasL expression was almost equal in both of these cells upon doxorubicin treatment as shown by RT-PCR (Fig. 3C), Western blot (Fig. 3C2), or
FIGURE 3. Effect of doxorubicin on induction of NF-κB and AP-1 in p53-negative and -positive cells. A, HCT116 (p53<sup>-/-</sup>) and wild type cells were treated with 1 μM doxorubicin for different times and harvested in lysis buffer. M, Marker lane for DNA ladder. Cell extracts were prepared. 300 μg of whole-cell extracts were run in a 9% SDS-PAGE gel. The gel was dried and exposed to a phospho-screen, and the radioactive bands were detected as activity of JNK. MCF-7 and U-937 cells, respectively. HCT116 (p53<sup>−/−</sup>) and HCT116 (Wild) cells (Fig. 3A1) were much more sensitive to doxorubicin than untreated cells. Oleandrin (OL) (100 ng/ml) was used to treat the cells as a positive control.

B, AP-1 DNA binding activity was assayed from nuclear extracts. NF-κB and AP-1 were used to detect NF-κB and AP-1 by gel shift assay. HCT116 (p53<sup>−/−</sup>) and wild type cells were treated with 1 μM doxorubicin for different times, and whole-cell extracts were prepared. 300 μg of whole-cell extracts were run in a 9% SDS-PAGE gel, and the gel was dried and exposed to a phospho-screen, and the radioactive bands were detected as activity of JNK. MCF-7 and U-937 cells, respectively (Fig. 3B1). The amount of FasL was measured from total RNA by RT-PCR. As shown in Fig. 4, A1 and A2, doxorubicin-mediated AP-1 DNA binding decreased gradually with the increasing concentrations of SP-600125. The SP-600125 did not alter p53 DNA binding in MCF-7 or HCT116 (Wild) cells (supplemental Fig. 4A). Doxorubicin induced 67% (p < 0.005) and 84% (p < 0.01) cell death in HCT116 (Wild) and HCT116 (p53<sup>−/−</sup>) cells, respectively. In anti-FasL Ab-pretreated cells, doxorubicin induced cell death 29 and 40% in HCT116 (Wild) and HCT116 (p53<sup>−/−</sup>) cells, respectively (Fig. 4B1). This result suggests that almost 40% cell death is protected by JNK inhibitor. In anti-FasL Ab-pretreated cells, doxorubicin induced cell death 28 and 29% in HCT116 (p53<sup>−/−</sup>) and HCT116 (Wild) cells, respectively (Fig. 4B2).

Inhibition of FasL Protects Doxorubicin-mediated Aggressive Cell Death in p53-negative Cells—To detect the role of FasL in aggressive cell death mediated by doxorubicin in p53-negative cells, both HCT116 (Wild) and HCT116 (p53<sup>−/−</sup>) cells were treated with different concentrations of SP-600125 (JNK inhibitor) and then stimulated with doxorubicin for 24 h. AP-1 DNA binding activity was assayed by gel shift assay. HCT116 (Wild) and HCT116 (p53<sup>−/−</sup>) cells were treated with 1 μM doxorubicin for different times, and whole-cell extracts were prepared, and the amount of PARP was measured from 100 μg of whole-cell extracts. Oleandrin (OL) (100 ng/ml) was used to treat the cells as a positive control.

As shown in Fig. 4, A1 and A2, doxorubicin-mediated AP-1 DNA binding decreased gradually with the increasing concentrations of SP-600125. The SP-600125 did not alter p53 DNA binding in MCF-7 or HCT116 (Wild) cells (supplemental Fig. 4A). Doxorubicin induced 67% (p < 0.005) and 84% (p < 0.01) cell death in HCT116 (Wild) and HCT116 (p53<sup>−/−</sup>) cells, respectively. In anti-FasL Ab-pretreated cells, doxorubicin induced cell death 29 and 40% in HCT116 (Wild) and HCT116 (p53<sup>−/−</sup>) cells, respectively (Fig. 4B1). This result suggests that almost 40% cell death is protected by JNK inhibitor. In anti-FasL Ab-pretreated cells, doxorubicin induced cell death 28 and 29% in HCT116 (p53<sup>−/−</sup>) and HCT116 (Wild) cells, respectively (Fig. 4B2). These data suggest that additive (almost 20%) cell death in HCT116 (p53<sup>−/−</sup>) cells is completely protected by anti-FasL Ab. As JNK inhibitor alone cannot block the additive cell death but anti-FasL Ab blocks it completely, we have looked at the possible other pathway for FasL expression, via IL-8 (17), in these cells. Both HCT116 (p53<sup>−/−</sup>) and HCT116 (Wild), when preincubated with anti-
FasL or -IL-8 Ab and SP-600125 followed by treatment with doxorubicin, demonstrated that anti-IL-8 Ab- and SP-600125-preincubated cells showed complete inhibition, but anti-FasL Ab- and SP-600125-preincubated cells showed partial inhibition of AP-1 DNA binding (supplemental Fig. 4B).

Anti-IL-8 Ab or SP-600125 partially but in combination completely inhibited FasL expression in both HCT116 (p53−/−) and HCT116 (Wild) cells as shown by the amount of FasL detected by Western blot (Fig. 4C1) and FasL-dependent luciferase activity (Fig. 4C2). These data suggest that expression
of FasL is dependent upon IL-8-mediated signaling and AP-1-dependent transcription. As IL-8 expresses FasL via NF-AT (17), the DNA binding activity of NF-AT was completely inhibited by anti-IL-8 Ab, but not by SP-600125, as shown by gel shift assay (Fig. 4D1) or NF-AT-dependent luciferase activity assay (Fig. 4D2). These data further suggest that both IL-8-mediated expression and AP-1-dependent FasL expression interplay in doxorubicin-mediated cell death. Doxorubicin induced 63% (p < 0.005) and 87% (p < 0.01) cell death in HCT116 (Wild) and HCT116 (p53−/−) cells, respectively. In SP-600125- or anti-IL-8 Ab-preincubated cells, doxorubicin induced cell death 29 and 40% in HCT116 (Wild) and HCT116 (p53−/−) cells, respectively. In a combination of SP-600125 and anti-IL-8 Ab, only 22% cell death was observed in both HCT116 (Wild) (Fig. 4E1) and HCT116 (p53−/−) (Fig. 4E2) cells. The amount of cleaved PARP also showed the similar extent of cell death as shown in the MTT assay (supplemental Fig. 4C). These data suggested that by inhibiting FasL, both p53-positive and p53-negative cells show a similar extent of cell death mediated by doxorubicin.

HCT116 (Wild) Cells Show High Basal Expression of K-ras, but Low Basal Expression of Fas—As FasL is expressed in both p53-positive and p53-negative cells upon doxorubicin treatment, the amount of Fas was measured to understand the differential cell death. A high basal amount of Fas was observed in HCT116 (p53−/−) cells, and doxorubicin did not alter this basal amount of Fas (Fig. 5A). The high basal amount of K-ras, but not H-ras, was observed in HCT116 (Wild) cells, as shown by Western blot (Fig. 5B1) and immunofluorescence (Fig. 5B2). Doxorubicin treatment did not alter this basal expression of K-ras. HCT116 (p53−/−) cells, transfected with vector, K-ras (wild), and K-ras (mutant) constructs were

FIGURE 5. Effect of doxorubicin in expression of K-ras and Fas on p53-positive and -negative cells. A and B1, HCT116 (p53−/−) and wild type cells were treated with 1 μM doxorubicin for different times, and whole-cell extracts were used to detect Fas (A) and K-ras (B1) by Western blot analysis. Blots were reprobed for tubulin. B2, untreated and doxorubicin-treated HCT116 (p53−/−) cells were transfected with vector, K-ras (wild), or K-ras (mutant) for 12 h. GFP-positive cells were counted from two independent experiments. C, cells were transfected with doxorubicin (1 μM) for different times, and then the amount of Fas and K-ras was assayed by Western blot. D, results are indicated as inhibition of cell viability in percent. E, HCT116 (p53−/−) and wild type cells were incubated with 1 μg/ml anti-Fas Ab for 2 h and then treated with doxorubicin (1 μM) for 72 h. Cell viability was assayed by MTT assay. F, cells were incubated with 1 μg/ml anti-Fas Ab for 2 h and then treated with doxorubicin (1 μM) for 72 h. FasL expression was assayed by the MTT assay and indicated as inhibition of cell viability.
treated with doxorubicin, and the amount of Fas and K-ras was measured. In K-ras (wild)-transfected cells, the amount of K-ras was observed (Fig. 5C, upper panel). The basal amount of Fas, which was detected in vector or K-Ras (mutant) cells, was almost completely inhibited in K-ras (wild)-transfected cells (Fig. 5C, lower panel). These data suggest that K-ras interferes with the basal amount of Fas, which is shown in p53-negative cells. K-ras-transfected HCT116 (p53−/−) cells showed a similar cell death as shown in HCT116 (Wild) cells, as shown by MTT assay (Fig. 5D). Anti-Fas Ab-pretreated cells showed 38% (p < 0.001) cell death in HCT116 (Wild) cells, but 75% (p < 0.005) cell death was observed in HCT116 (p53−/−) cells (Fig. 5E). Doxorubicin further potentiated cell death in both types of cells. In HCT116 (p53−/−) cells, transfected with K-ras showed similar cell death by anti-Fas Ab and doxorubicin as shown in HCT116 (Wild) cells. HCT116 (p53−/−) cells, transfected with vector or mutant K-ras, showed almost 75% cell death by anti-Fas Ab alone and almost 80% cell death by doxorubicin treatment. Co-incubation of anti-Fas Ab and doxorubicin showed almost 95% (p < 0.01) cell death in HCT116 (p53−/−) cells, transfected with vector or mutant K-ras (Fig. 5F). These data suggest that K-ras regulates the expression of Fas, which helps in differential cell death mediated by doxorubicin and anti-Fas Ab in p53-positive and -negative cells.

DISCUSSION

Doxorubicin is a widely used chemotherapeutic drug for treating breast cancer and many other cancers. Doxorubicin treatment often causes DNA damage, which leads to change in mitochondrial potential followed by cell death (3, 4). DNA damage often leads to activation of p53, which is a well known tumor suppressor. In many tumor cells, the p53 is non-functional either by deletion or by mutation of it. Doxorubicin is usually a good drug against breast tumor. Many studies are showing that might be a target of doxorubicin in breast tumor cells such as SKBr3 and MDA-MB-231 targeted by doxorubicin more aggressively (17). However, the role of p53 in tumor biology is very confusing. Chemotherapeutic drugs are effective even in the absence of p53. We have found that in p53-positive cells, doxorubicin-mediated cell death is slower and less potent than in p53 down-regulated cells, which is supported by a number of evidences such as conversion of MTT dye, nuclear and DNA fragmentation, LIVE/DEAD assay, caspase activation, and caspase-dependent cellular protein fragmentation. In p53-positive cells, activation of p53 and its dependent genes is observed, which always induces cell cycle arrest and thereby apoptosis. Although in p53-positive cells, apoptosis is observed, when compared with p53-negative cells, the apoptosis is 20−25% less at any time of doxorubicin treatment. It is surprising that p53-negative cells lack several p53-mediated genes that arrest cell cycle; still, doxorubicin-mediated cell death is much efficient. It is obvious that the unanswered question is that p53-mediated cell death might be proceeding through cell cycle arrest, but in p53-negative cells, the cell death may be independent of cell cycle arrest, and this needs to be studied further.

The p53 transcription factor activates several genes that usually help in cell cycle arrest. Some of the genes expressed by p53 are pro-survival genes such as Cox2, DDR2, EGF, etc. (29, 30). It has been reported that myosin VI, a p53-dependent gene that is involved in the endocytosis pathway, inhibits DNA damage in a p53-dependent manner (31). The high amount of p53 is also regulated by MDM2 (murine double mutant 2) through proteosomal degradation (32). However, all these reports suggest the down-regulation of p53. Although we did not observe the decrease in p53 activation, which was shown either by DNA binding activity or by p53-dependent gene expression, MDM2 helps proteosome-mediated degradation of p53. As we did not observe the decrease in the amount of p53 as shown by Western blot, the role of MDM2 in repression of doxorubicin-mediated cell death should be ruled out. It is important to look for the molecules that interact with p53 and its expression.

Doxorubicin is a well known inducer of caspases. Recruitment of proteins, such as Fas, leads to apoptosis. Recruitment of these proteins is often preceded by the activation of NF-κB via some receptors (such as FasL, TNF, and IFN-α (RAIL) with their respective receptors). It is known that co-stimulation of the extracellular signals such as cytokines, especially TNF and IFN-α, activate NF-κB (17). It has been reported that doxorubicin-mediated first phase activation of NF-κB leads to expression of FasL through a sequential process: NF-κB-dependent IL-8 expression, IL-8-mediated increase in intracellular free Ca2+, Ca2+-dependent calcineurin activation, calcineurin-mediated dephosphorylation of NF-AT, nuclear translocation of NF-AT, and NF-AT-dependent FasL expression (17). Suppression of FasL expression mediated by NF-κB (via IL-8) and AP-1 inhibits cell death almost 60% in p53-positive cells and 75% in p53-negative cells. Doxorubicin-mediated additive cell death (20−25%) in p53-negative cells is completely inhibited by suppression of FasL. These data suggest that FasL-mediated cell death plays a role in aggressive cell death in p53-negative cells induced by doxorubicin. Although FasL expression is observed in both p53-positive and p53-negative cells, the FasL-mediated response is still the determining factor for aggressive cell death in p53-negative cells. FasL interacts with its specific cell surface receptor Fas/CDD95 to induce cell death via caspase activation. To our surprise, higher basal expression of Fas is observed in p53-negative cells when compared with p53-positive
cells. The p53-negative cells showed more cell death upon exposure to anti-Fas Ab. These data further support that the expression of Fas in these cells is higher resulting in pronounced cell death, which is further potentiated by doxorubicin treatment. Mutant p53 has been shown to suppress Fas expression (38), which may not be true in the case of HCT116 cells where we are not detecting the basal expression of Fas.

We have also noticed that p53-positive cells have a high basal expression of K-ras. Ras family proteins are usually an activator of cell proliferation, but we are getting the opposite effect in p53-overexpressed cells. Basal K-ras expression is shown to be absent in colon cancer cells (9), which is supporting our observation that HCT116 cells have no basal expression of K-ras, whereas p53 overexpressed HCT116 cells show high basal expression of K-ras, which further supports that Ras is one of the p53-dependent genes (39). It has been reported that K-ras suppresses p53 through stabilization of Snail protein, which directly binds with p53, via expression of ataxia telangiectasia-mutated and Rad3-related proteins (40). How p53 regulates K-ras expression needs to be studied further. The Ras family of oncogenes, especially H-ras, has been shown to down-regulate Fas via the PI3 kinase pathway and thus execute an antiapoptotic effect (36). It has been reported that sustained activation of the Ras/Raf/MAPK cascade has thus execute an antiapoptotic effect (36). It has been reported that sustained activation of the Ras/Raf/MAPK cascade has been observed in activation of p53 (41). The possibility of basal expressed K-ras might be decreasing the amount of Fas in p53-positive cells.

Considering the usefulness of doxorubicin in different tumors, especially breast cancer, the detailed mechanism of action would help in finding drugs for effective therapeutic index and drug-resistance. We hypothesize that the doxorubicin-mediated cell death effect in p53 interplays in doxorubicin-treated cells where we are not detecting the basal expression of Fas. The mechanism of drugs in doxorubicin drug resistant, associated cell death effect in p53-positive cells would be useful to understand the drug resistance where K-ras puts a brake on cell proliferation, thereby slowing down apoptosis.

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Nuclear fragmentation data as detected by propidium iodide-stained cell nuclei supported the similar cell death in these cells (Fig. 1B). Doxorubicin-induced cell death 68% (p < 0.01), 76% (p < 0.005), 56% (p < 0.005), 80% (p < 0.01), and 72% (p < 0.005) in U-937, THP1, MCF-7, HeLa, and SKBr3 cells, respectively, at 72 h of treatment as detected by LIVE/DEAD assay (Fig. 1C).