p53 Promotes Selection for Fas-mediated Apoptotic Resistance

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ABSTRACT

Although p53 inactivation is implicated as a mechanism to explain diminished apoptotic response, it is clear that tumor cells that possess transcriptionally functional p53 can also be resistant to diverse apoptotic stimuli. We hypothesize that oncogenic activation and DNA damage are sufficient stimuli to increase the p53-dependent transcription of Fas and thereby establish a situation in which cell to cell contact could be a selective pressure to either lose p53 function or inactivate components of the Fas death pathway. Examination of genetically matched tumor cell lines that possessed either wild-type or null p53 loci indicated that cells possessing functional p53 increased their surface levels of Fas and Fas ligand (FasL) in response to DNA damage. In contrast, stress induced by changes in the tumor microenvironment such as decreased oxygen did not up-regulate Fas or FasL. Cells with wild-type p53 underwent Fas-mediated killing in the presence of either FasL-expressing killer cells or activating Fas antibodies, whereas cells in which p53 was deleted or inactivated were protected from such killing. Furthermore, Fas and FasL expression and induction became transcriptionally repressed in transformed cells with wild-type p53 with increasing passage, whereas other p53 downstream targets and functions, such as p21 inducibility and cell cycle arrest, remained intact. Repression of the Fas locus could be reversed by treatment with the histone deacetylase inhibitor trichostatin A. These results support a model of tumor progression in which oncogenic transformation drives tumor cells to lose either p53 or their Fas sensitivity as a means of promoting their survival and evade immune surveillance.

INTRODUCTION

Tumor escape from immune surveillance has been hypothesized to result from the inability of the immune system to react to the tumor (1). This failure to respond could be due to nonrecognition of tumor antigens, insufficient costimulation, anergy, tolerance, or immunosuppression (2). One system hypothesized to play a role in immune surveillance is the Fas-mediated killing of tumor cells by infiltrating lymphocytes (3–6).

Fas, also known as CD95 or APO-1, is a cell surface protein belonging to the tumor necrosis factor receptor family. Binding of FasL1 to a cell expressing Fas receptor results in its death by apoptosis (7). This interaction is thought to function in immune response termination, T cell activation-induced cell death, control of peripheral tolerance to self-antigens, and the maintenance of immune privilege in the eye and testes (8, 9). Whereas many tissues express the Fas receptor protein, FasL expression is limited to activated T lymphocytes, macrophages, and cells of the eye and testes (10, 11). Most recently, FasL has been reported to be expressed in human melanoma (12), hepatocellular carcinoma (13), and highly metastatic tumors of the colon (14). Expression of FasL on tumor cells has been hypothesized to function in deletion of infiltrating immune cells and thus aid in tumor escape from immune surveillance (15).

Several studies suggest that Fas transcriptional expression is p53 regulated (16–18). A p53-responsive element was identified within the first intron of the Fas gene and three putative p53-responsive elements are located within the promoter (16). In support of the direct transcriptional regulation of Fas by p53, K562 cells stably transfected with a plasmid containing a temperature-sensitive human p53 gene demonstrated a 4- to 6-fold up-regulation of cell surface Fas when p53 was in a wild-type conformation (19).

The clinical importance of the cross-talk between the p53 and Fas-FasL pathways in modulating apoptosis has been suggested by several studies (20, 21). Although numerous genotoxic therapies used in cancer treatment activate both p53 and Fas signaling pathways, the contribution of each pathway to cell killing is not well defined. Clearly, treatment of leukemic (20) and hepatocellular carcinoma cell lines (21) with chemotherapeutic agents causes up-regulation of FasL. In addition, hematoma cells have been shown to increase Fas surface expression in response to anticancer drugs (21), and incubation of blocking F(ab’2), anti-Fas antibodies protects these cells from doxorubicin- and bleomycin-induced apoptosis. However, in many of the same tumors in which Fas and FasL are activated, p53 is also activated and can signal cell death or cell cycle arrest. Tumors with mutated or deleted p53 have been reported to be resistant to radiation- and chemotherapy-induced apoptosis (22). It has been suggested that resistance to chemotherapy of p53 null tumors may in part be the result of their inability to elevate surface Fas in response to genotoxic stress (23). For example, human hepatomas with wild-type p53 were found to up-regulate Fas and FasL in response to chemotherapeutic drugs, whereas hepatomas in which p53 was mutated or deleted were resistant to chemotherapy-induced apoptosis and failed to up-regulate Fas and FasL (21).

Human cancers with a functional wild-type p53 genotype have also been shown to elevate their surface Fas expression in response to ionizing radiation (24). In contrast, human cancer cell lines in which p53 is mutated or deleted fail to demonstrate this response (24, 25). Given the evidence for cross-talk between p53 and Fas-FasL and the clinical importance of these pathways in cancer treatment, we examined Fas inducibility by genotoxic and nongenotoxic stimuli known to induce p53, in cell lines genetically matched, differing only in their p53 status.

In a survey of cell lines, those cells with wild-type p53 had greater surface levels of Fas and FasL and were more sensitive to FasL-mediated killing. Furthermore, we found that oncogenically transformed cells with wild-type p53 transcriptionally repressed the expression of Fas and FasL over time in culture. Oncogenic transformation therefore may establish a cellular context that leads to either loss of p53 or Fas expression during cell proliferation, protecting against Fas-mediated apoptosis during tumor expansion.

MATERIALS AND METHODS

Cell Lines. The following cell lines were used: MEF transformed with E1A/Ha-Ras, either wild-type or null for p53 (Scott Lowe, MIT, Cambridge, MA); MEF transformed with Myc, either wild-type or null p53 (Scott Lowe, MIT, Cambridge, MA); the human colorectal cancer cell line RKO and RC10.2
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Kathleen Cho, The Johns Hopkins University School of Medicine, Baltimore, MD; the human lung cancer cell line NCI-H1299 (lacking expression of the p53 protein) stably transfected with a tetracycline-inducible p53 construct; and lpr mouse myoblasts isolated from lpr mice transfected with either control vector or Fasl (Helen Blau, Stanford University, Stanford, CA). RC10.2 is a stable transfectant of RKO expressing the human papilloma virus 16 E6 gene to inhibit p53 function.

Cell Treatments. MEF, RKO, and NCI-H1299 cells were incubated in a hypoxic anaerobic chamber (Bactron Anaerobe Chamber, Cornelius, OR) at 0.01% O2 for varying time periods to determine the effect of hypoxia on Fas receptor induction and apoptosis. MEF, RKO, and NCI-H1299 cells were irradiated with 200-1000 eGy at a dose rate of 281 eGy/min from a 137Cs source.

FACS Analysis. To determine the surface levels of Fas and Fasl, on the MEFS, 1 × 106 cells were incubated on ice for 30 min with 1 µg/ml biotinylated anti-Fas antibody, Jo2 (BD PharMingen, San Diego, CA), anti-Fasl antibody, Kay-10 (BD PharMingen), or an irrelevant isotype control. After this incubation, the cells were washed and incubated on ice for 30 min with streptavidin-phycoerythrin (BD Immunocytometry Systems, San Jose, CA). The cells were then washed and analyzed with a FACScalibur flow cytometer (BD Immunocytometry Systems). This same protocol was followed with the RKO and NCI-H1299 cells. The antihuman Fas antibody was from BD Immunocytometry Systems and the antihuman Fasl antibody was NOK-1 (BD PharMingen). All experiments were conducted a minimum of three times.

Apoptosis Assays. Apoptosis was determined by staining cells with the APO-Direct Kit (PharMingen) followed by analysis on a FACScalibur flow cytometer (Becton Dickinson). The APO-DIRECT assay detects DNA strand breaks. Apoptosis was then confirmed by staining a subpopulation of all treated cells with Hoechst-propidium iodide followed by visualization on a fluorescent microscope. The activating anti-Fas antibody used to induce apoptosis in selected experiments was the antihuman clone DX2 (BD PharMingen). All experiments were repeated three times.

51Cr Release Assays. MEF or NCI-H1299 cells were plated at 10,000 per well of a 96-well plate. Cells were loaded with 1 µCi/well with 51Cr and allowed to incubate at 37°C for 2 h. After this incubation, the cells were washed three times with DMEM plus 10% FCS medium. After the last wash, 100 µl of fresh medium were added to each well. Myoblasts from lpr mice expressing Fasl or control vector were added to cells at ratios of 20:1, 10:1, and 1:1 in a final volume of 100 µl of medium. Medium (100 µl) was added to control wells measuring spontaneous release, and 100 µl of 0.1% Triton X-100 was added to wells measuring total lysis. Cells were allowed to incubate for 12 h and then centrifuged for 5 min at 1200 rpm. Supernatant (50 µl) was harvested from each well and counted on a β-scintillation counter. All treatments were done in triplicate, and experiments were repeated three times.

RNase Protection Assays. RNA was extracted with TRIzol reagent (Life Technologies, Inc., Grand Island, NY) according to the manufacturer’s direction. The extracted RNA was then used in a BD PharMingen mAPO-3 kit, according to the manufacturer’s instruction, to measure Fas mRNA levels. All RNase protection assays were conducted three times to confirm results.

Selection Experiments. The down-regulation of Fas inducibility as a function of cell proliferation was tested by analyzing surface levels of both Fas and Fasl in p53 wild-type and null MEFS over time in culture. MEF cells at passages 5, 10, 15, and 20 were tested both for their basal Fas and Fasl surface levels as well as their ability to up-regulate Fas and Fasl after 6 Gy irradiating radiation. Staining was repeated three consecutive times to confirm results.

The human lung cancer cell line NCI-H1299 stably transfected with a tetracycline-inducible p53 construct was incubated in either the presence or the absence of doxycycline (200 ng/ml) to induce p53 expression. These cells were then treated for 12 h with the activating anti-Fas antibody, DX2, or Fasl-expressing myoblasts to induce Fas-mediated apoptosis. These experiments were repeated three times to confirm results.

DNA Sequencing of p53. Total RNA was extracted from E1A/Ras-transformed MEF p53+/+ passage 5 and 20 cells with TRIzol Reagent (Life Technologies, Inc., Grand Island, NY), and reverse transcribed with oligo(dT) as primer to produce cDNA. Full-length p53 was PCR amplified from the above cDNA template with the following primers (5’-GGTTGACCCCT- TCTCCG; 3’-TACGTCCTGAGTGCAGGCC). The 1.2-kb p53 PCR product was separated on a 1% agarose gel and sequenced on an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Branchburg, NJ).

Cell Cycle Analysis. p53 wild-type and null MEF cells, passage 5, transformed with E1A/Ras as well as p53 wild-type MEF cells, passage 20, were exposed to 6 Gy ionizing radiation and collected at 12 and 24 h after irradiation. The cells were fixed in 70% ethanol at −20°C overnight. On the next day, cells were washed and resuspended in 0.5 ml of PBS containing 50 µg/ml propidium iodide and 5.0 µg/ml RNase A. The cell suspension was incubated at 37°C in the dark for 30 min and then analyzed on a FACScalibur flow cytometer. These experiments were repeated twice to confirm results.

Western Blotting Analysis of p21. Cell extracts were prepared from control and irradiated MEF cells at 3 and 6 h after 6 Gy ionizing radiation. Protein (50 µg) was loaded onto each lane of a 12% Tris-glycine gel. After electrophoresis, the proteins were transferred onto a polyvinylidene fluoride membrane. The membrane was blocked in 5% BSA + 5% nonfat milk + 0.1% Tween 20 in Tris-buffered saline and then incubated with antinouse p21 rabbit antisem (Greg Hannon, Cold Spring Harbor Laboratory) at a dilution of 1:2000 in blocking buffer for 1 h. The membrane was washed and then incubated with alkaline phosphatase-antirabbit IgG (Vector, Burlingame, CA) at a dilution of 1:3000 in blocking buffer. The blot was washed again and developed with enhanced chemiluminescence substrate (Amersham Life Science, Little Chalfont, United Kingdom). The fluorescent product was detected with a STORM Optical Scanner (Molecular Dynamics, Sunnyvale, CA).

Analysis of Fas and Fasl Expression in Late Passage Cells. Genomic sequencing of Fas and Fasl, was conducted to determine whether mutations occurred in these genes in the late passage wild-type MEFS. Protein surface expression and mRNA levels of expression were analyzed by FACS and RNase protection assay as described above. DNA acetylation was analyzed by comparing mRNA levels of Fas and Fasl in late passage cells in the presence and the absence of the histone deacetylase inhibitor, trichostatin A, administered at 25 ng/ml for 24 h.

RESULTS

We first set out to learn whether genotoxic and nongenotoxic stimuli known to induce p53, specifically ionizing radiation and hypoxia, would also induce Fas mRNA expression. p53 wild-type and null MEFS were exposed to either 6 Gy of ionizing radiation or 0.01% oxygen. RNA was collected at 0, 6, and 12 h after ionizing radiation and 0, 6, and 12 h after exposure to 0.01% oxygen (Fig. 1). Basal levels of Fas mRNA were 3-fold higher in wild-type p53 cells and were also found to be induced 4-fold after 6 Gy of ionizing radiation. Hypoxia, on the other hand, failed to up-regulate Fas in either wild-type or null p53-containing cells.

We next sought to determine whether the increased Fas message observed in p53 wild-type cells following radiation correlated with an increase in Fas expression on the cell surface. MEF cells were stained for Fas and analyzed by flow cytometry at 0 and 12 h after radiation or at 0, 8, and 20 h after hypoxia (Fig. 2). Consistent with the Fas mRNA data, ionizing radiation induced Fas levels in p53 wild-type cells while failing to induce surface Fas elevation in cells lacking p53. In contrast to genotoxic insults, hypoxia, a nongenotoxic stress known to induce p53, failed to induce Fas in these cells regardless of their
p53 status. This finding is significant in light of the recent report of Fas being induced on vascular smooth muscle cells transfected with a chimeric p53–4-hydroxytamoxifen-sensitive estrogen receptor, following 4-hydroxytamoxifen exposure (17). Thus, whereas previous studies suggest that Fas is induced by ectopic overexpression of wild-type p53, we demonstrate that not all stresses known to induce p53 also induce Fas.

We also examined the kinetics of Fas inducibility after ionizing radiation (Fig. 3). The basal surface levels of Fas were 7-fold greater in p53 wild-type cells and surface levels of Fas increased almost 4-fold over basal levels following ionizing radiation. This induction of surface Fas in p53 wild-type cells was also found to increase in response to increasing radiation dose (Fig. 3).

To learn whether this p53-dependent Fas induction in response to ionizing radiation was a generalized phenomenon, we tested other oncogenically transformed cell types that were genetically matched, except for their p53 status. Fas levels were measured in the human colorectal cancer cell line RKO and RC.10.2 (RKO cells stably transfected with the human papilloma virus 16 E6 protein to disrupt p53 function) as well as Myc-transformed MEFs that were genetically matched except for p53 status. Fas was induced by ionizing radiation in a dose-dependent manner that was substantially greater in wild-type p53 cell lines (Fig. 4).

Previous reports suggest that some cancer cells express FasL (1, 26). We examined whether p53 wild-type and null MEFs also expressed FasL and whether their surface levels increased in response to ionizing radiation (Fig. 5). Both p53 wild-type and null cells were found to express FasL. In addition, the levels of FasL were substantially elevated on the surface of cells with a wild-type p53 genotype in response to increasing radiation dose. This induction of FasL in response to radiation coupled with the elevation of surface Fas expression may provide an additional explanation as to why p53 wild-type cells and tumors are more sensitive to radiation-induced apoptosis. Thus, these results further support the concept that the p53 genotype of a tumor cell could make it more susceptible both to self-elimination through the Fas pathway and to increased sensitivity to Fas-mediated killing by the immune system.

To determine whether wild-type p53 was able to sensitize cells to FasL-mediated killing, in vitro 51Cr release assays were conducted (Fig. 6). FasL-expressing myoblasts isolated from lpr mice (Fas receptor null) were used as death effector cells, and the MEFs were used as the target cells. Fas-expressing p53 wild-type MEFs were sensitive to Fas-mediated killing, and this killing was found to increase after exposure to ionizing radiation. MEF cells lacking p53 were protected from Fas-mediated killing both in their resting state and in response to ionizing radiation.

The ability of p53 status of a cell to affect its sensitivity to Fas-mediated apoptosis was further tested with the use of a human...
lung cancer cell line lacking p53 stably transfected with a tetracycline-inducible p53 construct. With doxycycline to induce p53, it was found that these cells underwent Fas-mediated apoptosis when incubated in the presence of an activating Fas antibody but were protected from this killing in the absence of p53 induction by doxycycline (Fig. 7). The increased apoptosis observed in these cells in the presence of doxycycline and activating Fas antibody was greater than what could be explained by activation of p53 alone.

Finally, we noticed that p53 wild-type MEFs exhibited reduced basal levels of apoptosis as well as reduced apoptotic sensitivity to radiation with increasing passage in culture (Fig. 9A) and wondered whether this loss in sensitivity could be attributed to changes in surface levels Fas and FasL. The down-regulation of Fas and FasL inducibility as a function of cell proliferation (27) was tested by analyzing surface levels of Fas and FasL over time in culture (Fig. 8). Indeed, surface levels of Fas and FasL decreased with increasing passage. In addition, the surface induction of Fas and FasL after radiation diminished in MEFs with increasing passage. This down-regulation of Fas and FasL was most pronounced in the p53 wild-type cells, and the null cells were more stable in their maintenance of radiation-induced elevation of FasL.

In attempt to understand the findings of diminished apoptotic sensitivity and Fas and FasL expression in the late passage p53 wild-type cells, sequence analysis was conducted to confirm that these cells still possessed wild-type p53 (data not shown). Late passage cells were indeed found still to maintain a wild-type p53 genotype. We next set out to learn whether the p53 in these cells was functional by studying their ability to undergo cell cycle arrest and induce p21 protein after ionizing radiation (Fig. 9, B and C). In contrast to the loss of Fas and FasL surface expression as well as apoptotic sensitivity seen in the late passage wild-type p53 cells, other p53 downstream targets and functions remained intact (Fig. 9). Late passage cells were shown to possess an intact G1 cell cycle arrest that prevented increases in cell ploidy after ionizing radiation (Fig. 9B) as well as maintaining their ability to induce the cyclin-dependent kinase inhibitor p21 (Fig. 9C). Because the cells were transfected with the adenovirus E1A gene that inhibits Rb, the p53-modulated G1 arrest is abrogated (28). Thus, the loss of Fas and FasL surface expression is not due to loss of p53 functionality, but in Fas and FasL expression and processing pathways.

Although Fas was found to be wild-type, we hypothesized that loss of its expression may be due to transcriptional repression. To test this hypothesis, we incubated late passage MEFs in the presence of the histone deacetylase inhibitor, trichostatin A (25 ng/ml for 24 h), which relieves transcriptional repression caused by histone acetylation. Late passage p53 wild-type MEFs were stained for their surface expression of Fas and FasL after ionizing radiation in the presence and absence of trichostatin A (Fig. 10A). Trichostatin A restored basal surface levels of Fas and FasL (Fig. 10B) as well as their ability to be elevated after ionizing radiation.

To confirm that the loss of Fas and FasL in late passage cells was due to transcriptional repression that could be reversed with tricho-
statin A treatment, RNase protection assays were performed on late passage MEFs before and after ionizing radiation in the presence and absence of trichostatin A (Fig. 10B). Late passage wild-type MEFs were indeed found to have greatly reduced levels of Fas and FasL message which failed to be induced in response to ionizing radiation. In contrast, late passage wild-type MEFs treated with trichostatin A had elevated basal levels of Fas and FasL message and induced these genes in response to ionizing radiation. This effect was found to be specific to Fas and FasL in that message levels of FADD remained unchanged after trichostatin A treatment.

DISCUSSION

The physiological significance of cross-talk between Fas and p53 has been controversial (29). Data obtained from gene knockout studies suggests that one pathway is not necessary for the function of the other. Apoptosis in response to activating Fas antibodies is not impaired in liver or thymus cells isolated from p53 knockout mice, nor is Fas-mediated activation-induced death of lymphocytes (30). Furthermore, caspase-8 (the initial caspase activated in the Fas signal transduction cascade) knockout mice are not protected against etoposide-induced apoptosis, a genotoxic stress known to induce p53 (31). On the other hand, data obtained from oncogenically transformed cells suggest that the two pathways somehow interact. The Fas gene has been demonstrated to be a transcriptional target of p53 and tumors with wild-type p53 have been reported to up-regulate Fas and activate caspase-8 in response to genotoxic stress. Given the importance of p53 in signaling apoptosis and the Fas pathway in immune regulation, we addressed this controversy by testing genetically matched tumor cell lines varying only in their p53 status for their ability to modulate Fas in response to genotoxic and nongenotoxic stress. In human and mouse cell lines tested in this study, cells with wild-type p53 had significantly greater basal surface levels of Fas than did their genetically matched counterparts in which p53 was inactivated or absent. The increase in surface Fas expression in cells with wild-type p53 results in an increased sensitivity to FasL-mediated killing both in response to activating Fas antibodies and in response to FasL-expressing killer cells. Our results clearly demonstrate that the p53 status of a cell regulates the level of Fas expression. Importantly, loss of p53 was found to protect oncogenically transformed cells from Fas-mediated apoptosis. Thus, in terms of the tumor expansion, our results suggest that inactivation of p53 would be one means of protecting the tumor from immune-mediated elimination.
Of interest was our finding that radiation, but not hypoxia, was capable of inducing an increase in Fas surface expression in cells with wild-type p53. Although both stresses are known to induce p53, only radiation resulted in an elevation of Fas surface expression. This result suggests that Fas is not a major contributor to p53-dependent apoptosis induced by hypoxia in the tumor microenvironment.

Loss of Fas expression in tumors has been shown to occur during malignant progression (32). We likewise found that p53 wild-type MEFs lost Fas as well as FasL surface expression and inducibility during proliferation in culture by transcriptionally repressing these genes. In expressing elevated levels of both Fas and FasL, early passage cells, by their very proximity, are under a selective pressure to lose either p53 or their ability to induce Fas as a means of escaping “self-induced” apoptosis and in the process become more apoptotically resistant. These results suggest that even under situations in which p53 is capable of transactivating gene expression, Fas desensitization may occur by transcriptional silencing. In support of this hypothesis, late passage p53 wild-type MEFs lost Fas as well as FasL surface expression and inducibility with increasing passage number. Cells lacking p53, on the other hand, were more stable in maintaining their surface FasL levels. Results are representative of data obtained from three independent experiments.

Complimentary to our results, a recent report described the pivotal role of cross-talk between Fas/FasL and p53 in the etiology and metastasis of UV radiation-induced skin tumors (18). Keratinocytes from FasL-deficient mice exposed to UV radiation exhibited significantly less apoptosis coupled with a greatly enhanced frequency of p53 mutations than keratinocytes from wild-type mice. It was hypothesized that the survival of the p53-mutated skin cells exposed to UV radiation was due to their inability to be removed by the Fas pathway. We likewise found that in the process of tumor progression, a selective pressure may exist on the cell to either lose or mutate p53 or down-regulate Fas as a means of promoting survival and evading immune surveillance.

This selective desensitization of the Fas pathway during cell proliferation appears to be a specific p53 adaptation. Late passage MEFs, although exhibiting greatly reduced surface levels of Fas and FasL, as well as inducibility, still possess an intact p53-mediated G2 cell cycle arrest and are capable of inducing p21 following ionizing radiation. Of note, this loss of Fas induction with increasing passage failed to occur in the advanced passage p53 wild-type cancer cell line RKO (data not shown). In that RKO cells do not express surface FasL (data not shown), they are not under selective pressure in culture as are the MEFs, which need to inactivate either p53 or Fas to escape self-apoptotic elimination. Our results demonstrate that oncogenically transformed cells, with wild-type p53, are under a selective pressure during proliferation to down-regulate their sensitivity to Fas-mediated apoptosis (which might have already occurred in the advanced passage cell line RKO with the loss of surface FasL). These data suggest that in the process of tumor progression, a selective pressure may exist on the cell to either lose or mutate p53 or down-regulate Fas as a means of promoting survival and evading immune surveillance.

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Fig. 8. Surface Fas and FasL expression in MEF cells over time in culture. A. MEF cells at passages 5, 10, 15, and 20 were stained for surface Fas expression in their resting state and after exposure to 6 Gy ionizing radiation. Cells with wild-type p53 underwent selection in culture, down-regulating both their basal levels of Fas and their radiation inducibility of this receptor. B. MEF cells at passages (p.) 5, 10, 15, and 20 were stained for basal surface FasL expression as well as expression after exposure to 6 Gy ionizing radiation. Cells with wild-type p53 greatly down-regulated their basal levels of FasL as well as their radiation inducibility of FasL with increasing passage number. Cells lacking p53, on the other hand, were more stable in maintaining their surface FasL levels. Results are representative of data obtained from three independent experiments.

Fig. 9. Assessment of p53 function in early passage (p.5) and late passage (p.20) p53 wild-type MEF cells. A. percentage of apoptosis in MEF cells after 6 Gy ionizing radiation. Passage 20 MEF cells exhibit a >4-fold decrease in apoptosis 24 h after 6 Gy ionizing radiation. B. cell cycle arrest in MEF cells 12 h after 6 Gy ionizing radiation. Both passage 5 and passage 20 MEF cells arrest after 6 Gy ionizing radiation. C. p21 induction in MEF cells at 0, 3, and 6 h after exposure to 6 Gy ionizing radiation. p21 protein was induced slightly over 3-fold in both passage 5 and passage 20 p53 wild-type MEFs after ionizing radiation. Although late passage p53 wild-type MEF cells demonstrate a diminished apoptotic response to genotoxic stress, the p53 functions of cell cycle arrest and p21 induction remain intact. Results are representative of data obtained from three independent experiments.
In terms of cancer treatment, these results demonstrate that not only are p53 null cells more resistant to radiation-induced apoptosis but they also up-regulate FasL in response to genotoxic stress without increasing Fas expression. A potential outcome of such treatment could be the elimination of immune surveillance cells that possess Fas receptors with the retention of, and thus enhanced survival of, the tumor. In terms of experimental investigation of the relationship between p53 and the Fas pathway, our data stress the importance of using early passage cells in the exploration of the p53 and Fas relationship and caution against the use of late passage oncogenically transformed cells in studying the intersection of these two signaling pathways.

The applicability of these results to the tumor microenvironment still must be tested in vivo. However, our results provide yet another possible mechanism of how loss of p53 aids in tumor survival; i.e., by promoting the loss of surface Fas and Fas inducibility, elimination of p53 could enhance tumor survival through evasion of immune surveillance.

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