Modulation of DNA damage-induced apoptosis by cell adhesion is independently mediated by p53 and c-Abl

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Conventional cancer therapies are based on preferential killing of tumor cells by DNA damage. Previous work showed that, for certain cell types, loss of integrin-mediated adhesion decreased the apoptotic response to DNA damage because of decreased p53 levels after detachment from the extracellular matrix. Integrin ligation restored p53 and sensitivity to DNA damage. In this study, we show that c-Abl mediates a second pathway by which adhesion to extracellular matrix regulates cell killing by chemotherapeutic agents 5-arabinofuranosylcytosine, cisplatin, and camptothecin. Activation of c-Abl tyrosine kinase by DNA damage requires cell adhesion. Abl-dependent stabilization of p73, a p53-related proapoptotic transcription factor, is also adhesion-dependent. Sensitivity to the Abl inhibitor STI571 suggests differential utilization of the p53 and c-Abl/p73 pathways by different tumor cell lines. These data suggest that killing of p53-negative tumor cells by chemotherapy would be enhanced by integrin ligation to activate the alternative c-Abl/p73 pathway.

Current cancer therapies are based primarily on radiation and chemotherapy that damage DNA to selectively kill fast-growing tumor cells. This strategy is efficacious in the treatment of childhood leukemia and solid tumors at early stages, but suffers from the limitation that a small number of cancer cells commonly evade therapy and accumulate additional mutations, leading to recurrence of therapy-resistant tumors. The tumor suppressor gene p53 provides a key pathway that mediates cell death after DNA damage (1, 2). DNA damage increases the level and the transcriptional activity of p53, resulting in induction of cell cycle arrest genes such as $p21^{Waf1}$ and proapoptotic genes such as Noxa, Puma, and Bax. Inactivation of p53 is one of the most common genetic alterations in human cancer and is associated with progression to metastatic disease, resistance to therapy, and genomic instability.

We recently described an effect of integrin-mediated adhesion on p53 levels and subsequent sensitivity of cells to DNA damage (3). For susceptible cell types, loss of adhesion to extracellular matrix (ECM) caused a decrease in p53 levels, leading to decreased apoptosis after exposure to ionizing radiation or chemotherapeutic drugs. The change in p53 was caused by a more rapid decline in levels of p19Arf, which inhibits MDM2 to prevent ubiquitin- and proteosome-dependent degradation of p53. This behavior was cell type specific. It was observed in a melanoma, a rhabdomyosarcoma and a fibrosarcoma line, as well as primary mouse embryonic fibroblasts (MEFs). However, several other tumor cell types either died directly after detachment or showed no decrease in sensitivity to DNA damage in suspension.

A second pathway that mediates cellular responses to DNA damage involves the c-Abl tyrosine kinase. Activation of c-Abl by DNA damage contributes to cell cycle arrest and apoptosis via the p53 homolog p73 (4–6). Interestingly, c-Abl is also regulated by integrins (7, 8). Detachment of cells from the ECM leads to a decrease in c-Abl kinase activity and retention of c-Abl in the nucleus. Replating cells on ECM triggers exit of c-Abl from the nucleus, transient localization to nascent focal adhesions, and increased kinase activity (7, 9). This dual role of c-Abl as a mediator of both integrin signaling and the cellular response to DNA damage led us to investigate its involvement in adhesion-dependent apoptosis. We report here that c-Abl provides an alternative or parallel pathway by which integrins influence the response to DNA damage in susceptible cells. We also show that decreased sensitivity to DNA damage does not require complete detachment, but that conditions of reduced adhesion that permit spreading and migration significantly decrease apoptosis after DNA damage.

Materials and Methods

Cells. MEFs were maintained in DMEM supplemented with 10% FCS. HT1080 fibrosarcoma cells were maintained in DMEM supplemented with 10% FCS, nonessential amino acids, and Hepes-buffered saline. C4-2 and DU145 prostate carcinoma and M21L melanoma cells were maintained in DMEM supplemented with 10% FCS. Cells detached from the substratum by mild trypsinization were resuspended in medium containing 0.8% carboxymethylcellulose to inhibit cell aggregation. To induce apoptosis, cells were treated with 5-arabinofuranosylcytosine (araC), *cis*-diamminedichloroplatinum (II) (cisplatin), or camptothecin at varying concentrations for the indicated times.

Migration Assays. MEFs grown to near confluence in DMEM with 10% serum were trypsinized and replated in DMEM with 0.5% serum on tissue culture plastic dishes that had been coated with the indicated concentrations of fibronectin (FN) then blocked with 10 mg/ml heat-denatured BSA. After 2 h, dishes were rinsed to remove unattached cells and then placed on a microscope stage in an environmental chamber that maintains temperature, CO_2, and humidity (10). Migration was followed by taking time lapse images for 4 h, then quantified by single cell tracking as described (10).

Apoptosis Assays. For the analysis of caspase-3 activation, cell lysates were prepared in 50 mM Hepes, pH 7.4, containing 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 0.1 mM EDTA, and 1 mM DTT. Samples were assayed in lysis buffer supplemented with 100 mM NaCl and 10% glycerol by using the colorimetric substrate Asp-Glu-Val-Asp (DEVD)-*p*-nitoanilide with or without the caspase-3 inhibitor Ac-DEVD-CHO (Alexis Biochemicals, San Diego). Background activity (in the presence of inhibitor) was subtracted to obtain specific caspase-3 activity.

Terminal deoxynucleotidyltransferase-mediated UTP end labeling (TUNEL) assays were performed by using the labeling kit from Roche Molecular Biochemicals according to the manufacturer's instructions. MEFs were transfected with GFP-GAP

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Abbreviations: MEF, mouse embryonic fibroblast; ECM, extracellular matrix; araC, 5-arabinofuranosylcytosine; FN, fibronectin; TUNEL, terminal deoxynucleotidyltransferase-mediated UTP end labeling.

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(generous gift from Dr. K. Moriyoshi, Kyoto University, Kyoto) by using Effectene (Qiagen, Chatsworth, CA) according to manufacturer instructions. Fluorescence from GFP-GAP (GTPase-activating protein) is membrane associated and retained in apoptotic cells, and is therefore a convenient marker for scoring. Cells were scored by fluorescence microscopy.

Western Blotting. Cells were lysed in buffer containing 158 mM NaCl, 10 mM Tris (pH 7.4), 0.1 mM EGTA, 1% Triton X-100, and 1% sodium deoxycholate and 0.1% SDS containing protease inhibitors: 10 mM NaF, 2 mM sodium vanadate, 1 mM PMSF, and 10 μg/ml each aprotinin and leupeptin. Equal amounts of protein were separated by SDS/PAGE, transferred to nitrocellulose, and probed with primary and secondary antibodies in 5% nonfat dry milk in Tris-buffered saline. Blots were visualized with enhanced chemiluminescence (Amersham Pharmacia).

Abl Kinase Assay. Cells seeded at 2×10^6 per 15-cm plate were used 24 h later. They were left on the dish or suspended in 1% methyl cellulose for 90 min, then treated with araC for 30 min. Cells were chilled on ice, rinsed with PBS, and suspended in 150 μl (3× cell volume) of hypotonic buffer (10 mM β-glycerophosphate/1 mM EDTA/1 mM EGTA/0.1 mM sodium vanadate/2 mM MgCl2/10 mM KCl/1 mM DTT/0.5 mM PMSF/10 μg/ml aprotinin and leupeptin). After 30 min on ice, cells were disrupted in a micro dounce homogenizer with 20 strokes. The homogenate was layered on top of a cushion of 1 M sucrose in hypotonic solution and centrifuge at 4,500 rpm (Beckman microcentrifuge) for 15 min at 4°C to pellet the nuclei. The nuclei were washed three times with 1 ml of 1 M sucrose/hypotonic and resuspended in lysis buffer (50 mM Trism pH 7.5/5 mM EDTA/150 mM NaCl/1% Nonidet P-40/0.1% Brij35/1 mM DTT/10 mM β-glycerophosphate/2 mM sodium vanadate/1 mM NaF, 2 mM PMSF/10 μg/ml aprotinin and leupeptin). After 30 min on ice, they were sonicated (30 pulses), spun for 15 min at 14,000 rpm in a microfuge at 4°C. To the supernatants was added 2.8 μg of purified K12 antibody, tubes were rotated for 4 h at 4°C, and complexes collected on 10 μl of protein A-Sepharose for 1 h at 4°C. Immune complexes were washed successively with 1 ml of lysis buffer with 500 mM NaCl, 250 mM NaCl, 150 mM NaCl, and then kinase buffer (50 mM Tris, pH 7.5/10 mM $MgCl_2$/1 mM DTT). Immune complexes in 30 μl of kinase mixture (2 μg of GST-Crk-CTD/1 mM ATP) were incubated at 30°C for 30 min. Samples were extracted with 20 μl of 3× SDS sample buffer and analyzed by SDS/PAGE and Western blotting with 8E9 (3 μg/ml) to detect Abl and with the anti-phosphotyrosine antibody 4G10 to detect phosphorylated GST-Crk-CTD.

Generation of Cells from p53/abl-Double Knockout Mice. Compound heterozygous mice ($abl^{+/-}p53^{+/-}$) were generated by mating $abl^{+/-}p53^{+/+}$ (11) and $abl^{+/+}p53^{+/-}$ (12) mice. Embryonic and neonatal lethality associated with the $abl^{-/-}$ genotype was not rescued by p53 mutation, because $abl^{-/-}p53^{+/-}$ and $abl^{-/-}p53^{-/-}$ mice exhibited this early lethality phenotype (I. C. Hunton and J.Y.J.W., unpublished data). Heterozygosity at the Abl locus also did not affect the lifespan of the p53 mutant mice. These results were consistent with a lack of genetic interaction between the mouse abl and p53 genes. Primary MEFs were prepared from day 12 embryos and genotyped. MEFs from littermates were used in this study. At least two independently derived MEFs cultures of each genotype were used in the experiments.

Growth Rate. Primary MEFs were treated with AraC either on tissue culture plastic or in 1% methylcellulose for 48 h under the exact conditions used to measure apoptosis. At the end of the treatment, cells were washed extensively and then divided among four 96-well plates. Each sample was plated in duplicates per plate. Twofold serial dilutions of the plated cells were then made on each plate. At days 2, 4, and 6 after plating, cells were washed twice with PBS, and then stained with 0.1% crystal violet in 50% methanol for 30 min. After extensive washing with PBS, the dye was extracted with 10% acetic acid and the dye concentration was determined by reading the OD units at 600 nM with a microplate reader. To calculate doubling time, growth curves were fit to an exponential curve, $R^2 > 0.96$ in all cases.

Induction of p73. Human p73α was stably expressed in p73-null 3T3 fibroblasts through retroviral-mediated gene transfer. Cells were preincubated adherent to tissue culture plastic (attached) or suspended in 1% methylcellulose (suspended) with STI571 for 2 h and then treated without or with araC (10 μM, 2 h) or *cis*-diamminedichloroplatinum (II) (CDDP) (25 μM, 6 h). Cells were collected, lysed in RIPA buffer (50 mM Tris·HCl, pH 7.4/1% Nonidet P-40/0.25% sodium deoxycholate/0.1% SDS/150 mM NaCl/1 mM EDTA/1 mM NaF/1 mM sodium pervanadate/10 mM β-glycerolphosphate/1 μg/ml aprotinin/1 μg/ml leupeptin/1 μg/ml pepstain/1 mM PMSF), sonicated (20 pulses at output level 5, with duty cycle of 50%), and then boiled in SDS sample buffer. Approximately 70 μg of total protein was used in SDS/PAGE (8% gel), and probed with affinity-purified anti-p73 polyclonal antibodies (G. Sun and J.Y.J.W., unpublished data). A nonspecific band in the 35-kDa range of the gel was used as loading control.

Results

Dependence of Apoptosis on FN Coating Density. We showed previously that nonadherent fibroblasts or tumor cell lines had diminished sensitivity to DNA damage (3). Although there is evidence that tumors *in vivo* shed large numbers of cells into the circulation (13–16), matrix proteolysis and invasion by tumor cells may more commonly decrease adhesive strength without causing complete detachment (17). We therefore sought to test whether decreased levels of adhesion affected sensitivity to DNA damage. Cells were plated on coverslips coated with concentrations of FN ranging from 0.1 to 10 μg/ml. After 2 h, dishes were rinsed to remove nonadherent cells and araC was added. Cells spread on all of these surfaces. Apoptosis in response to DNA damage was assessed after exposure to araC by using the TUNEL assay. Fig. 1*A* shows that apoptosis in response to araC was highest at 10 μg/ml FN and decreased by up to 3-fold at lower FN coating densities. Control experiments showed that cells that detached during the incubation showed a similar dependence on FN coating density; more cells detached at lower FN densities, but fewer were apoptotic. By contrast, cells on low doses of FN migrated faster, with the highest rate observed at a coating density of 0.3 μg/ml (Fig. 1*B*). Thus, conditions of reduced adhesion that maintained spreading and accelerated migration substantially decreased cell death induced by DNA damage.

Effects on Cell Growth. These as well as previous results (3) show that integrin-mediated adhesion increases apoptosis in response to DNA damage; however, DNA damage can also induce growth arrest, which may make a significant contribution to the therapeutic response (18). To test whether nonadherent cells preferentially growth arrest instead of undergoing apoptosis, adherent or suspended cells were treated with 16 μM araC for 48 h, then replated on tissue culture plastic in fresh medium, and cell growth was assayed for 6 days. As expected, the number of adherent cells that initially replated was decreased by 74 ± 2%, whereas the decrease in suspended cells was only 20 ± 13%. In adherent cells, those that survived araC proliferated at a rate that was similar to the untreated cells (not shown). For suspended cells, previous exposure to araC induced somewhat

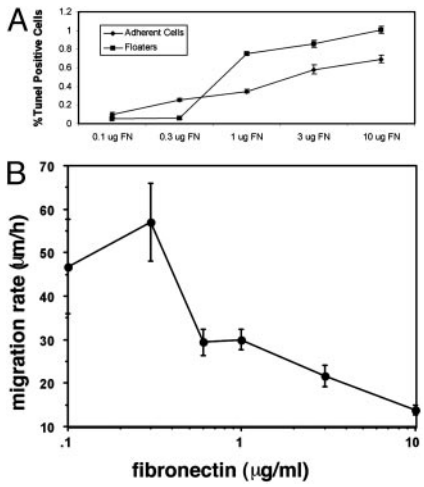

Fig. 1. Dose–response curve for FN. (*A*) MEFs were plated on different concentrations of FN for 2 h, and the dishes were rinsed to remove nonadherent cells. Cells were then treated with 20 μM araC. After 24 h, cells were fixed and stained by using the TUNEL assay. TUNEL-positive cells were scored. In some experiments, floating cells were collected separately and analyzed. Low concentrations of FN had greater numbers of floaters, but levels of apoptosis were lower. Values are means ± SEM from three independent experiments. (*B*) MEFs were plated in low serum on tissue culture dishes coated with FN at the indicated concentrations. After 2 h, dishes were rinsed and adherent cells were followed by time lapse imaging. Rates of migration were calculated for ≈40 cells in three independent experiments. Values are means ± SEM.

slower growth (doubling time of 2.3 days for untreated vs. 3.0 for treated cells). These results showed that araC did not cause irreversible growth arrest in either the adherent or the suspended populations. Thus, growth arrest was a relatively minor component of the response to araC. These results show that suspended cells are genuinely resistant to DNA damage under these experimental conditions.

Independent Requirements for p53 and c-Abl. We previously found that integrin-mediated adhesion regulates p53 via its upstream regulator p14/p19 Arf (3). Although loss of p53 in these studies resulted in a large decrease in apoptosis after DNA damage, we wished to consider the possibility that additional integrin-sensitive but p53-independent pathways might exist. In the published experiments, MEFs showed diminished sensitivity to araC when nonadherent. These experiments also demonstrated that caspase 3 as an indicator of cell death gave results comparable to TUNEL assays and to analysis of cell morphology. We therefore used caspase 3 activity to compare WT MEFs (Fig. 2*A*) to $p53^{-/-}$ MEFs (Fig. 2*B*). Although the $p53^{-/-}$ cells required higher doses of araC and showed a lower extent of death even at saturation, apoptosis was still dramatically higher when cells were adherent.

To test the involvement of c-Abl, WT and $p53^{-/-}$ MEFs were treated with STI571, an inhibitor of Abl kinase. WT MEFs exposed to STI571 showed a partial inhibition of cell death (≈50%), with the observed caspase activity remaining adhesion dependent (Fig. 2*C*). $p53^{-/-}$ MEFs treated with STI571 became virtually resistant to DNA damage-induced apoptosis whether adherent or suspended (Fig. 2*D*).

To confirm the contribution of c-Abl to adhesion-dependent sensitivity to DNA damage, cells derived from p53 and abl double knockout embryos were treated with araC and assayed for caspase-3 activity. These results show that cells lacking both p53 and c-Abl were highly resistant to araC under all conditions, whereas cells from $p53^{-/-}abl^{+/+}$ littermates showed adhesion-dependent death as before (Fig. 3*A*). Cells treated with camptothecin or cisplatin, two other chemotherapeutic agents that trigger DNA damage via distinct mechanisms, showed a similar pattern of abl- and adhesion-dependent killing (Fig. 3 *B* and *C*). Taken together, these data provide strong evidence that c-Abl represents a second, p53-independent pathway for adhesion-mediated cell death in response to DNA damage.

Adhesion-Dependent Activation of c-Abl Kinase by araC. The c-Abl tyrosine kinase is activated by both cell adhesion (7, 8, 19) and DNA damage (20) In unpublished studies, we have found that

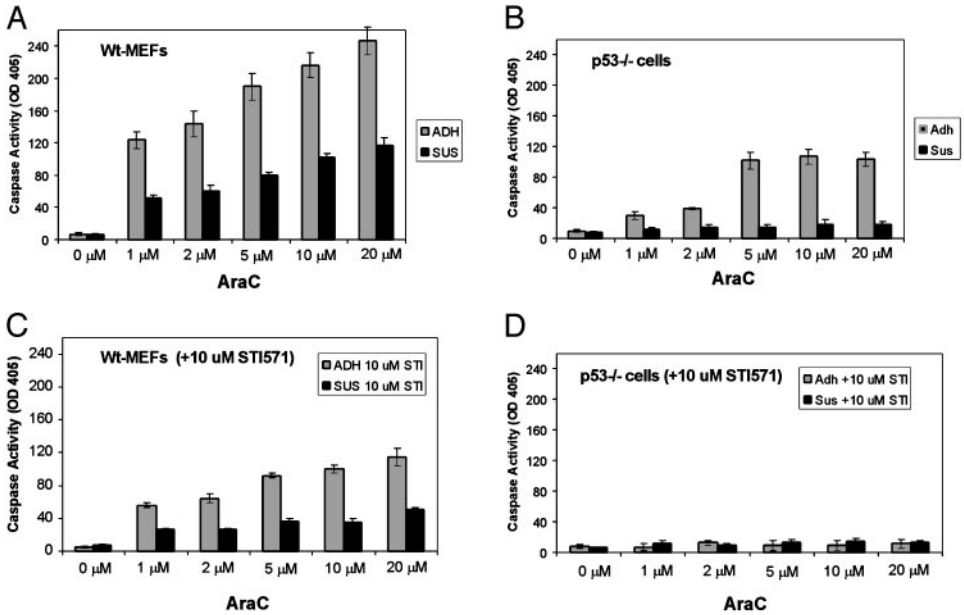

Fig. 2. p53 and c-Abl contribute to araC-induced apoptosis. MEFs were left adhered to tissue culture plastic or detached and kept in suspension. At 2 h araC was added, and after 48 h caspase 3 activity was assayed. (*A* and *B*) WT and $p53^{-/-}$ MEFs, respectively, were analyzed without STI571. (*C* and *D*) The same cells were analyzed in the presence of 10 μM STI571. Values are means ± SEM from three independent experiments.

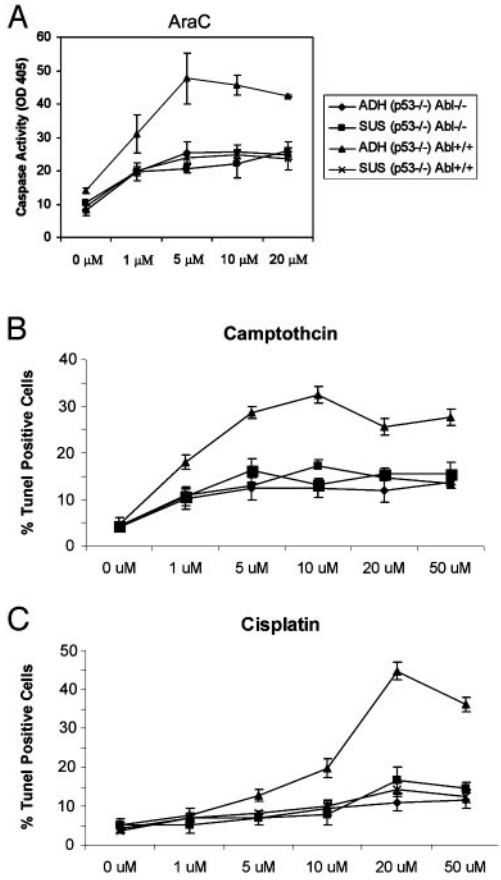

Fig. 3. Analysis of $p53^{-/-}$ and c-$Abl^{-/-}$ cells. MEFs isolated from $p53^{-/-}$/$abl^{+/+}$ or $p53^{-/-}$/$abl^{-/-}$ mice were left adhered to tissue culture plates or detached and kept in suspension. At 2 h, the indicated chemotherapeutic agent was added, and at 48 h caspase 3 activity or TUNEL stain was assayed. Values are means ± SEM from three independent experiments.

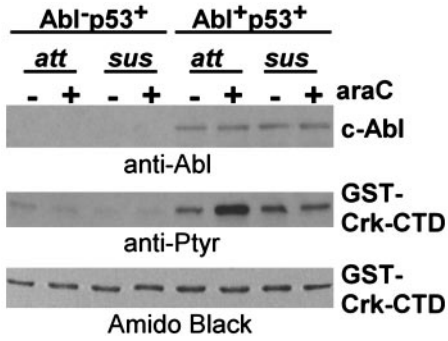

Fig. 4. Abl kinase activity. MEFs from WT or $Abl^{-/-}$ mice were left adherent to tissue culture plastic (att) or detached and kept in suspension (sus). At 2 h, cells were treated with 10 μM araC for 30 min, and c-Abl was immune precipitated. Tyrosine kinase activity toward a fusion protein containing GST and the C-teminal domain of crk was then assayed by Western blotting with antiphosphotyrosine antibody. The GST-crk protein was visualized by amido black staining of the nitrocellulose. Similar results were obtained in three independent experiments.

ionizing radiation activated the nuclear c-Abl tyrosine kinase in adherent but not in suspended fibroblasts (R. Baskaran and J.Y.J.W., unpublished observation). Because activation of the nuclear c-Abl tyrosine kinase by DNA damage is associated with the induction of apoptosis (21), we examined the activation of c-Abl tyrosine kinase by araC in adherent and suspended MEFs. When adherent cells were exposed to araC, we observed an increase in nuclear Abl kinase activity (Fig. 4), as reported (16). Activation of c-Abl by araC occurred equally well in $p53^{+}$ (Fig. 4) and $p53^{-}$ (data not shown) MEFs, ruling out a requirement for p53 in the process. By contrast, nuclear Abl isolated from nonadherent cells showed little change in activity after exposure to araC. This result demonstrates that cell adhesion is required for activation of c-Abl by DNA damage

Abl induction of apoptosis is mediated in part through stabilization of the p53 homolog p73 (4–6). To test the role of p73 in this process, we examined the effects of cell adhesion on accumulation of p73. Western blotting showed that DNA damage by araC or cisplatin increased p73 levels in adherent but not suspended cells, and that induction was blocked by STI571 (Fig. 5*A*). Thus, p73 induction correlates with Abl kinase activity. Additionally, $p73^{-/-}$ cells were less sensitive to araC compared with WT MEFs (Fig. 5*B*). Unlike WT cells, $p73^{-/-}$ MEFs showed no further decrease in apoptosis in the presence of STI571 (not shown). These results are consistent with a role for p73 downstream of c-Abl in the induction of apoptosis in this system.

Induction of p53 by araC Is Independent of c-Abl. To further test whether p53 and c-Abl act independently in the integrin dependent cell death pathway, p53 protein levels were examined. Cells were exposed to 10 μM STI571 overnight, and p53 protein levels in adherent and detached MEFs were analyzed by Western blotting. We observed that p53 was ≈50% lower after treatment with STI571. However, loss of adhesion resulted in a similar decline in p53 with and without STI571 (Fig. 6). These data indicate that c-Abl contributes to basal p53 levels, but that the effect of cell adhesion on p53 is independent of c-Abl. To confirm this pharmacological result, p53 levels in $Abl^{-/-}$ MEFs were assayed. Detachment from the ECM triggered a decrease in p53 similar to that seen in $Abl^{+/+}$ cells (data not shown).

Cell Type Specificity. These data indicate that two distinct pathways mediate effects of cell adhesion on DNA damage-induced

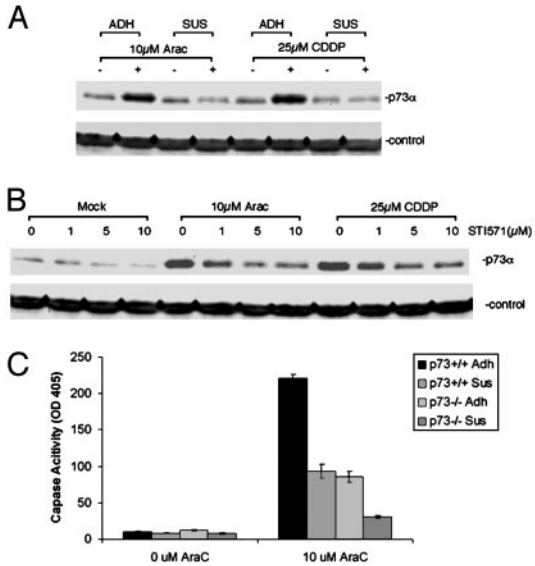

Fig. 5. Role of p73. (*A*) Adherent or suspended cells were treated with araC or cisplatin (CDDP) as indicated, and p73 levels were assayed by Western blotting. A nonspecific band at 35 kDa was used as a loading control. (*B*) Cells were treated with araC or cisplatin in the presence of the indicated concentrations of STI571, then p73 was assayed as in *A*. (*C*) Apoptosis of $p73^{-/-}$ and WT MEFs after 48 h with or without 10 μM araC was compared by assaying caspase-3 activity. Both cell types are $p53^{+/+}$. Values are means ± SEM ($n = 3$).

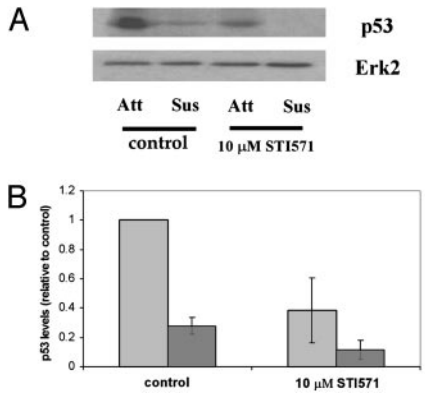

Fig. 6. Effects of adhesion on p53 do not require c-Abl. MEFs left untreated or incubated in 10 μM STI571 for 18h were analyzed by Western blotting for p53 protein levels. Erk2 was assessed as a control for protein loading. (*A*) Western blot. (*B*) Quantification of p53 levels normalized for Erk2. Values are means ± SEM from three independent experiments.

cell death. To probe the importance of these pathways, two tumor cell types, HT1080 fibrosarcoma and M21 human melanoma cells were examined. Treatment with araC in the presence of STI571 caused only a slight decrease in death of HT1080 cells (Fig. 7*A*). By contrast, STI571 treatment of M21L melanomas almost completely blocked apoptosis (Fig. 7*B*). These findings suggest that c-Abl makes little contribution to apoptosis in response to DNA damage in fibrosarcoma cells but is the primary apoptotic pathway in this melanoma. These results also support the notion that effects of p53 and c-Abl are largely independent.

We also examined two prostate carcinoma cell lines, DU145 and C4-2 (22). We observed that DU145 cells died within 48 h after being placed in suspension without exposure to araC (not shown). This result is typical of many anchorage-dependent cell types and is thought to represent a mechanism that prevents metastasis (23, 24). C4-2 cells, however, survived in suspension and, when treated with araC, showed decreased sensitivity when nonadherent (Fig. 7*A*). STI571 blocked caspase activation in adherent C4-2 cells by >60%, suggesting that a major fraction of the apoptosis pathway depended on c-Abl.

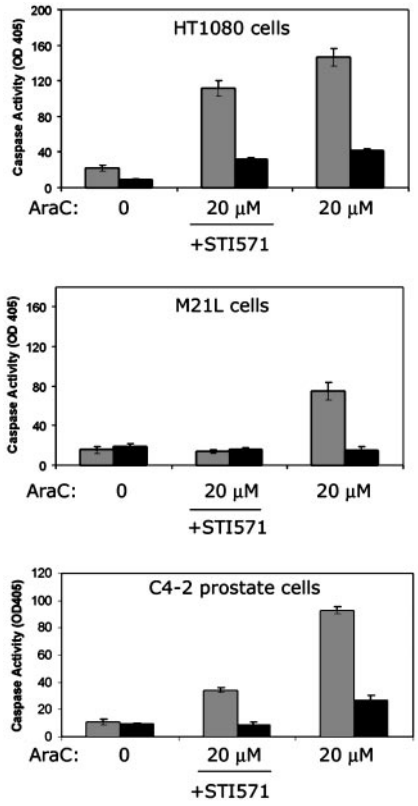

Fig. 7. Cell type specificity. HT1080 fibrosarcoma, M21L melanoma, or C4-2 prostate carcinoma cells were left adherent on the dish (gray bars) or detached and kept in suspension (black bars). Cells were treated with the indicated concentrations of araC with or without 10 μM STI571 as indicated. Caspase-3 activity was assayed at 48 h. Values are means ± SEM from three independent experiments.

Discussion

Our previous work (3) showed that when fibroblasts and certain tumor cells were detached from the ECM and kept in suspension, apoptosis in response to DNA damage was decreased because of decreased levels of p19Arf and p53. In this paper, we found that complete detachment from the ECM is not required for diminished sensitivity to DNA damage, because cell death in response to araC was reduced in cells on lower FN coating densities. These results may be relevant to tumor cells *in vivo*, where adhesiveness may be diminished because of degradation of the ECM or down-regulation of cell adhesion via other pathways such as changes in integrin expression (25, 26) or integrin affinity (27). This result suggests that changes in tumor cell phenotype that fall short of complete detachment may decrease sensitivity to chemotherapy. We also found that when cells were treated with araC in suspension and then replated, growth potential after DNA damage was only slightly diminished. This result excludes the possibility that suspended cells preferentially growth arrest instead of undergoing apoptosis.

We next found that p53 does not fully account for the effect of cell adhesion on sensitivity to DNA damage. Although p53-null cells were much less susceptible to araC, the residual cell death remained adhesion-dependent, indicating the existence of at least one other pathway. Inhibition of c-Abl kinase activity or genetic deletion of c-Abl blocked killing of p53-null MEFs. Additionally, activation of the c-Abl tyrosine kinase by araC required cell adhesion. Two other DNA damaging chemotherapeutic agents, cisplatin and campothecin, gave similar results. Stabilization of p73 correlated with c-Abl activity and $p73^{-/-}$ cells showed lower levels of apoptosis after DNA damage, supporting a role for p73 downstream of c-Abl. Activation of Abl kinase in the nucleus is sufficient to trigger apoptosis (28, 29). Thus, c-Abl appears to mediate the second, adhesion-dependent death pathway. These two pathways appear to be largely independent, as Abl kinase activity was not affected by the presence of p53 and the effect of adhesion on p53 levels was retained in the absence of c-Abl protein or activity. Under conditions where both p53 and c-Abl were inhibited or genetically deleted, cells were highly resistant to DNA damage-induced apoptosis.

Examination of several cell lines yielded the surprising result that different cell types show differential sensitivity to STI571, suggesting that they rely on c-Abl to varying degrees. In MEFs, c-Abl accounts for close to 50% of cell death, with p53 presumably mediating the rest. M21L melanoma cells, by contrast, appear to depend primarily on c-Abl for apoptosis, whereas HT1080 fibrosarcoma cells showed almost no effect of STI571. Examination of two prostate lines showed that DU145 cells died in suspension in the absence of DNA damage, whereas LNCAP C4-2 cells, in which this control mechanism was absent, showed adhesion- and c-Abl-dependent cell death. It should be noted that STI571 is not completely specific for c-Abl tyrosine kinase, hence, its effect on apoptosis could be caused by the inhibition of other kinases. However, results with the Abl-deficient MEFs

strongly support a role for c-Abl in linking cell adhesion status to DNA damage-induced apoptosis.

These results are of particular importance in connection with loss of p53 during tumor progression. Many metastatic tumor cells have mutations in the p53 pathway (2). However, our data show that the c-Abl pathway can still confer adhesion-dependent sensitivity to DNA damage in such cells. We previously found that adding divalent anti-integin antibodies to suspended cells could stimulate the integrin pathway and increase sensitivity to DNA damage (3). Therefore, stimulation of integrins, in conjunction with radiation or chemotherapy, would be predicted to enhance therapeutic effectiveness even in metastatic tumors with mutant p53. These results substantially broaden the possible range of tumors for which integrin stimulation might be an effective adjunct therapy.

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