Calibrizing at E2F binding sites on promoter regions to act as a transcriptional repressor and recruit of histone acetylase. The region of the RB protein responsible for E2F family binding is termed the large pocket and spans amino acids 379–870 (1, 2). Control of RB binding to E2F family members is exerted during late G1 phase of the cell cycle by cyclin D in association with cyclin-dependent kinases 4 and 6 (3). Maintenance of RB phosphorylation is carried out by other kinase such as cyclin E-cdk2 (4). RB phosphorylation is also affected by p16INK4A, an inhibitor of cdk4-cdk6, an important tumor suppressor in human cancer (5, 6).

RB control over the cell cycle can be interrupted by binding to the transforming proteins of DNA tumor viruses such as SV40 large T antigen, adenovirus E1A, or HPV E7. The viral proteins share a common LXCXE amino acid motif that binds to the A/B pocket of RB extending from amino acids 379–772 (7–9). The binding site of the proteins is located in the B box (amino acids 646–772) and is stabilized by the A box (9, 10). The A/B pocket, which is the site for a large number of activating RB mutations in human tumors, is also a site for binding of cellular proteins. Therefore, the A/B pocket is likely to play an important role in RB tumor-suppressor functions (2, 11).

In addition to its role in cell cycle regulation, RB influences the function of at least two proteins that are important in control of cell death, the c-Jun N-terminal kinase (JNK) and late activation of ABL kinase. In addition, p38 MAPK activation occurred independent of RB. ABL kinase, JUN N-terminal kinase, and p38 MAPK activity were all required for RB-mediated DU-145 cell death after γ-irradiation.
vitro but conferred sensitivity to radiation-induced cell death (38, 39). At 72 h after irradiation, we observed 30–50% apoptosis of DU-145 cells stably transfected with RB. However, caspases associated with the intrinsic cell death pathway were not activated, and expression of intact RB persisted after irradiation. Irradiation-induced apoptosis in DU-145 cells expressing RB was insensitive to zVAD but was blocked by serine protease inhibitors (39). This protocol describes the induction of RB with signaling pathways during DU-145 cell death induced by γ-irradiation. The data show that, in the appropriate cellular milieu, RB may play a role in death-signal transduction.

MATERIALS AND METHODS

Cell Culture—Growth of DU-145 cells and derivation of RB-expressing cell lines has been previously described (39). DU-2.16 cells were derived in our lab by similar procedures. DU-2.16 and DU-3.12 were two of several clones selected for sustained growth and retention of RB expression during prolonged passage. Cells were irradiated with a JL Sheepard Mark I Irradiator. UV exposure was done at 60 J/m² 48 h after transfection with GFP and RB expression plasmids. Cell death was assayed 14 h after UV exposure.

Plasmids and Transient Transfection Experiments—RB expression plasmids have been described previously (40, 41). DU-145 cells and RB-2.16 cells were transfected with appropriate expression vectors and with a green fluorescent protein (GFP) expression vector using SuperFect™ transfection reagent (Qiagen). Cells were exposed to γ-radiation 48 h after transfection. Immediately before assay for apoptosis, cells were sorted into a parallel group of cells were treated with corresponding transcriptional inhibitors in the absence of radiation. Cells were then incubated with 20 μM of total protein was incubated with 20 μl of immobilized phospho-p38 MAP kinase monoclonal antibody/protein A/G beads at 4 °C overnight. After rinsing with lysis buffer and kinase buffer sequentially, the pellet was suspended in 50 μl of 1% kinase buffer with 200 μM ATP and 2 μg of activating transcription factor-2 (ATF)-2 fusion protein and incubated overnight at 4 °C. P38 MAP kinase activity was assayed by Western blot with anti-phospho-ATF-2 antibody at dilution of 1:1,000.

ABL tyrosine kinase activity was analyzed by peptide phosphorylation assay. Cells were co-transfected with GFP and appropriate expression vectors. GFP-positive cells were lysed with 1× cell lysis buffer as described above. Anti-CBL immunoprecipitation was performed by adding 400 μg of total protein and 2.5 μg of anti-ABL antibody (24–11) (Santa Cruz Biotechnology) for 2 h at 4 °C and adding 30 μl (50%) of protein A/G for another 1 h at 4 °C. Beads with immune complexes were washed with kinase buffer and incubated with 100 μg of protein A/G for another 1 h at 4 °C. The homogenates were separated into cytosolic and membrane fractions by ultracentrifugation. Equal amount of protein were subjected to Western blot analysis using anti-cytokine c antibody (Santa Cruz Biotechnology) and anti-β-actin antibody (Sigma).

DNA Laddering—After drug exposure, pelleted cells (2 × 10⁶ cells) were lysed in 1% Nonidet P-40, 20 mM EDTA, 50 mM Tris-HCl, pH 7.4, kept on ice for 10 min, and centrifuged for 10 min at 1600 ×.g. SDS was added to the supernatant (10%) to a final concentration of 1%, and RNase A was added to a final concentration of 200 μg/ml. The mixture was then incubated for 2 h at 37 °C. Reaction was added with proteinase K to a concentration of 200 μg/ml and incubated for 2 h at 56 °C. DNA was isolated in 20 mM Tris-Cl buffer (10 mM Tris-Cl, 1 mM EDTA) and run on a 1% agarose gel in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0).

RESULTS

Cell Death Mediated by RB—We extended our observations of the effect of RB on DU-145 cell death by deriving additional DU-145 clones expressing RB. Newly derived DU-2.16 cells had a very similar death response to γ-irradiation compared with the previously described DU-1.1 cells and B5 cells. Although a low level of DNA fragmentation could be seen after irradiation, no low molecular weight DNA fragments (“laddering”) were seen (Fig. L4). We were not able to demonstrate activation of caspases in the intrinsic cell-death pathway (Fig. L1B). We previously showed that DU-145 cells are able to undergo robust caspase activation during apoptosis induced by okadaic acid.
Fig. 1. RB effects on DU-145 cell death. A, agarose gel demonstrating DNA fragmentation 72 h after exposure to the indicated dose of γ-radiation. TSU-Pr1 cells provide the positive control. B, Western blotting to detect activated caspases 3, 7, and 9. DU-lux and DU-2.16 cells were treated with 20 Gy of irradiation and harvested at 0, 24, and 48 h after irradiation. Positive control cells treated with okadaic acid are shown at the right. C, Western blotting of subcellular fractionated cell extracts to detect cytochrome c egress into the cytoplasm. Cells were treated as shown and harvested 48 h after treatment. At the time of harvest cells treated with okadaic acid showed ~40% apoptosis. D, Western blot showing RB expression and degradation at various time points after exposure to 20 Gy of irradiation. At 8 h caspase cleavage of RB can be seen in TSU-Pr1 cells. By 24 h, all of the RB in TSU-Pr1 cells has undergone cleavage. E, E2F1 promoter activity at different time points after exposure of cells to 20 Gy of irradiation. F, apoptosis of DU-145 cells 72 h after exposure to UV irradiation. Cells were transfected with the indicated plasmids. Apoptosis assay was done on GFP-positive cells.
and therefore had an intact caspase cascade (39). We were able to detect a small increase in cytoplasmic cytochrome c in DU-2.16 cells after irradiation, suggesting that the intrinsic mitochondrial-death pathway had been activated (Fig. 1C). We previously showed that RB-mediated cell death could be blocked by serine protease inhibitors such as N^\text{\textregistered}-tosyl-l-lysylcholoromethyl ketone (TLCK), but not by caspase inhibitors like z-Val-Ala-Asp (OMe)-CH2F (zVAD) (39).

The endogenous RB protein has antiapoptotic effects and is a target for caspase cleavage in most cells (27, 48–54). For example, irradiation of TSU-Pr1 cells is known to activate caspases that cleave RB (Fig. 1D). In contrast, RB is not cleaved after irradiation of B5 cells (39) or of DU-2.16 cells, perhaps due to the minimal level of caspase activation. Fig. 1D shows that RB was present in both phosphorylated and unphosphorylated states in DU-145 transfected cells and that irradiation caused some dephosphorylation of RB within 8 h of exposure. The truncated RB in DU-145 cells is expressed at low levels and is difficult to detect with Western blotting (39). As an index of RB activity, we assayed a reporter construct under the control of an E2F1-responsive promoter. Baseline activity of the promoter in DU-lux cells exceeded activity in DU-2.16 cells. Irradiation had no effect on the E2F1-responsive promoter, consistent with the finding that phosphorylation of RB was not appreciably affected by irradiation of DU-145 cells (Fig. 1E). We previously showed that γ-irradiation did not substantially affect cell cycle distribution of DU-145 cells that did or did not express wild-type RB (39). Finally, the proapoptotic effect of RB in DU-145 cells may be restricted to damage caused by γ-irradiation since RB expression inhibited DU-145 cell death after UV exposure (Fig. 1F).

We assayed the expression of candidate pro- and antiapoptotic proteins whose expression has been reported to be affected by RB and, therefore, could contribute to the effect of RB on apoptosis of irradiated DU-145 cells. The growth factor IGF-I has been shown to act as a survival factor and can be regulated by E2F1 (55–57). We also assayed the expression of the inhibitory binding protein IGFBP3, which can have proapoptotic effects (58). The overall level of IGFBP3 was lower in B5 cells than in DU-lux cells and, interestingly, increased after irradiation, but not until 48 h (Fig. 2A). We also examined the levels of AKT and phosphorylated AKT in DU-lux and B5 cells. This was done by immunoprecipitating AKT and then Western blotting the immunoprecipitated with either AKT antibody or antibody specific for phosphorylated AKT. We saw no differences in levels of AKT or AKT phosphorylation at the time of irradiation or at 48 h after irradiation in either cell line (Fig. 2B). APAF1, the proapoptotic mediator of death signaling by cytochrome c egress from the mitochondria, can have altered expression due to RB (59). However, we found no differences in baseline expression of APAF1 in our control or RB-transfected cell lines (Fig. 2C). Finally, we analyzed expression of P73, a proapoptotic protein whose expression is controlled by E2F1 and ABL (60–64). Although we found higher baseline p73 expression in DU-145 than the transfected cells, there was essentially no detectable p73 in DU-lux, B5, and DU-3.12 (Fig. 2D). The increased baseline P73 expression in DU-145 cells did not correlate with cellular response to γ-irradiation because neither DU-145 nor DU-lux cells underwent apoptosis, but B5 and DU-3.12 cells did.

**Mutations That Affect Interactions of RB with Other Proteins**

**RB Mediation of Apoptosis**

**Mutations That Affect Interactions of RB with Other Proteins Attenuate Its Proapoptotic Effects**—RB is a multifunctional regulator of transcription factors and of the transcriptional complex. RB can bind to E2F-1 and inhibit E2F-1-mediated transcription (65–67). RB can also form a transcriptional repression complex by binding and recruiting molecules such as histone deacetylase I to E2F-1-binding promoter regions (68–70). We used mutants of RB to analyze RB functions important for its proapoptotic effects (Fig. 3A). These experiments were conducted by transient cotransfection of the RB-expression vector and a GFP selection marker that was used to isolate transfected cells by sorting (see “Materials and Methods”). To demonstrate that the various mutant RB constructs used in these experiments were expressed in DU-145 cells, we performed transient transfection along with green fluorescent protein-selectable marker and analyzed RB expression in the transfected cells. As shown in Fig. 3B, six RB plasmid expression vectors engendered protein expression in DU-145 cells.

**RB mutant 13S lost the ability to bind to ABL but retained interaction with E2F and inhibition of cell growth and colony formation** (40). The 13S construct conferred sensitivity to DU-145 cell death (Fig. 3C). This result suggested that retention of E2F-1 binding and regulation of cell growth were consistent with the proapoptotic affect of RB. In contrast, PSM.9I and PSM.2S both had minimal effects on cell death (Fig. 3C) (41). The PSM.2S mutation eliminates serines 807 and 811, thus conferring constitutive ABL binding and loss of Rat-1 growth suppression, but retaining regulation of E2F-1 binding by phosphorylation (41, 71). The PSM.2S construct, however, retains the ability to modulate the phenotype of SAOS-2 cells and is more resistant to the effects of cyclin A overexpression than wild-type RB (71). The PSM.9I mutant has constitutive binding to E2F-1 and ABL and retains growth suppression of Rat-1 cells (41). The RB mutant RB(N757F) has been shown to elim-
inicate binding of the A/B pocket to proteins with LXCXE motifs such as histone deacetylase I and the ability to induce cell-growth arrest, but retain E2F-binding (42). The RB(N757F) mutant was unable to mediate cell death and was as devoid of proapoptotic activity as PSM.9I. The compound mutant PSM.9I(RB)N757F also had no effect on cell death. It thus appeared that disruption of binding to histone deacetylase I or constitutive binding to ABL resulted in loss of the proapoptotic functions of RB. Both PSM.2S and PSM.9I were able to compete with wild-type RB and block the mediation of DU-145 cell death (Fig. 3D). PSM.2S and PSM.9I both have constitutive ABL binding. PSM.9I, which cannot be phosphorylated, was a more effective competitor of apoptosis.

The Role of ABL in RB-mediated Apoptosis—Because RB mutants with constitutive ABL binding were competitive inhibitors of DU-145 cell death, we studied the role of ABL in DU-145 cell apoptosis induced by γ-irradiation. The ABL tyrosine kinase, a target for RB binding, is activated by γ-irradiation and is proapoptotic when localized to the nucleus (13, 18, 72). Overexpression of ABL itself in DU-145 cells predisposed to radiation-induced cell death but to a lesser degree than wild-type RB. A kinase-dead ABL-K290R

![Fig. 3](image-url)

**Fig. 3. Effects of RB mutant constructs on DU-145 cell death.** A, maps of RB protein produced by the constructs used in these experiments (40, 41). B, Western blot of RB expression in DU-145 cells transfected with different expression vectors and selected for GFP expression. The β-actin control Western blot is shown below for each sample. C, apoptosis of transiently transfected DU-145 cells 72 h after exposure to 20 Gy of irradiation. The constructs used are indicated below the histogram. D, apoptosis of DU-2.16 cells that were transiently transfected with the indicated expression plasmids. Transfection was carried out so that the cells were exposure to a constant amount of DNA. Cells were sorted for the cotransfected selectable marker GFP immediately before assay for apoptosis 72 h after 20 Gy of irradiation.
had no effect on cell death (Fig. 4A). Moreover, increasing expression of ABL could overcome the antiapoptotic effects of PSM.2S, consistent with the notion that PSM.2S was inhibiting apoptosis by complexing with ABL (Fig. 4B). Activation of ABL kinase was induced rapidly by γ-irradiation of DU-145 cells even though the parental cells did not undergo apoptosis after irradiation. In the presence of RB expression, ABL kinase underwent a second increase 24–48 h
after irradiation (Fig. 4C). The inset in Fig. 4C shows that levels of endogenous ABL were not altered by irradiation or by the presence of RB. The interaction of ABL and RB was further analyzed by coexpression of the two in transient transfection experiments. Whereas overexpression of ABL by itself was permissive for a low to moderate degree of cell death, RB alone facilitated a higher level of apoptosis, and coexpression of ABL enhanced the effect of RB (left side of Fig. 4D). RB constructs PSM.2S and PSM.9I with constitutive ABL binding had a dominant-negative effect on ABL and abrogated the modest degree of cell death enhancement mediated by ABL alone, consistent with the data in Fig. 4B. Furthermore, kinase-dead ABL had a dominant-negative effect that blocked the propapoptotic effect of RB. ABL kinase has both nuclear localization and nuclear export sequences that mediate shuttling of the ABL protein between nucleus and cytoplasm (13). Nuclear ABL kinase is activated...
by radiation via ATM to induce p73 in the process of mediating radiation-induced cell death (60, 73, 74). Removal of the ABL nuclear export sequence restricts the BCR-ABL oncoprotein to the nucleus and is proapoptotic (75). In DU-2.16, ABL with a disrupted nuclear export sequence is as propapoptotic as wild-type ABL, but ABL restricted to the cytoplasm due to loss of a nuclear localization sequence has reduced apoptotic activity (Fig. 4E). To confirm that the effects seen were caused by expression of the wild-type and mutant ABL vectors, we showed that each vector engendered similar levels of ABL expression in DU-145 cells (Fig. 4F).

**Fig. 6. Downstream effects of RB after irradiation.** A, assay for JNK activity after irradiation of the cell lines as shown. B, inhibition of cellular transcription by two different agents blocks RB-mediated cell death after exposure to 20 Gy of irradiation. C, ABL kinase activity in DU-2.16 cells transiently transfected with the indicated expression vectors. Cell extracts were generated and assayed 48 h after exposure to 20 Gy of irradiation as shown. Cells were transfected with the indicated plasmids and selected for GFP-positive status prior to the cell death assay.

**Interaction of RB and JUN—**Execution of cell death requires the activity of the c-Jun N-terminal kinase (JNK) (20). We previously showed that apoptosis mediated by RB involved the activation of JNK (39), whereas under other circumstances, RB binds to JNK to block its activity (19). RB can also interact directly with the transcription factor JUN via the JUN leucine zipper (76). We analyzed mutants of JUN in RB-mediated cell death (Fig. 5A). Overexpression of JUN by itself had no effect on cell death (left side of Fig. 5B). However, JUN overexpression slightly enhanced RB-mediated death, and a JUN transactivation mutant TAM-67, lacking amino acids 1–67 of the N-terminal transactivation domain, exerted an inhibitory effect on cell death (Fig. 5B). Similarly, the TAM-67 mutant had an inhibitory effect when cotransfected with ABL in DU-2.16 cells (left side of Fig. 5C). The TAM-67 JUN mutant had an additive antiapoptotic effect when transfected in DU-2.16 cells with kinase-dead ABL, suggesting that RB-JUN interaction contributed to cell death independent of ABL (right side of Fig. 5C). Mutations of the JUN transactivation (63/73) and protein-protein interaction leucine zipper domain (LZM-1) had dominant-negative effects on cell death. However, disruption of the DNA-binding domain (DBM-3) had no effect on cell death (Fig. 5D). Fig. 5E shows that the different JUN expression plasmids were expressed in transfected cells with protein species seen at estimated sizes predicted by the mutant plasmid constructs. Degradation fragments were also detected.

JNK is activated during cell stress and activates JUN by phosphorylating serines 63 and 73 (21). Activation of JNK in DU-145 cells occurred within 8 h of irradiation as shown in Fig. 6A. A low level of JNK activation occurred in DU-lux cells, but this activation was insufficient to induce cell death. In contrast, activation of JNK after irradiation of DU-2.16 cells was easily detected. Activation of JNK affects JUN and its targets for transcriptional control. To explore whether the permissive effect of RB expression on cell death was due to effects on transcription, we added low doses of either of two transcriptional inhibitors, rifamycin SV or β-5,6-dichlorobenzimidazole riboside, to DU-145 cells after irradiation. Either agent was able to block cell death 72 h after irradiation. Rifamycin SV was well tolerated by the cells. At higher doses, β-5,6-dichlorobenzimidazole riboside exposure was toxic by itself (Fig. 6B).

We showed above that irradiation of DU-145 cells induced a low level of ABL kinase activation nearly immediately that was followed at 48 h by further activation of ABL kinase only in the presence of RB (Fig. 4C). Early ABL kinase activation occurred...
independent of JNK activation. Consistent with this finding, ABL kinase activity at 24 h was not affected by the expression of the JUN63/73 even though JUN63/73 inhibited apoptosis (Figs. 5 D and 6 C). Taken together, the data show that ABL kinase activity was necessary but not sufficient for cell death and was independent of JNK activity and JUN phosphorylation.

Finally, we studied the activation of the cytoplasmic p38 mitogen-activated protein kinase (p38 MAPK) in the response of DU-145 cells to γ-irradiation (77–81). In particular, p38 MAPK has been implicated in both MYC-dependent cell death and caspase-independent death of HeLa cells exposed to photodynamic therapy (82, 83). As an example of a kinase whose activation was not dependent on RB, we demonstrated, by substrate phosphorylation, that P38 MAPK was activated in DU-145 cells after γ-irradiation irrespective of RB expression or cell death (Fig. 7 A). Inhibition of p38 MAPK by either of two inhibitors, SB202190 and SB203580, had no effect on JNK activity in irradiated DU-2.16 cells (Fig. 7 B), but diminished cell death (Fig. 7 C). Therefore, p38 MAPK contributed to cell-death signaling, but did not initiate death pathway activation and was not involved with JNK activation.

DISCUSSION

Our findings are summarized in the schema shown in Fig. 8. In the absence of RB expression, γ-irradiation of DU-145 cells activates several signaling pathways that result in activation of JNK, ABL kinase, and p38 MAPK. However, the intensity of the signals is insufficient to execute the cell death program. In the presence of RB expression, JNK activation is markedly enhanced and ABL kinase activation undergoes later activation. p38 MAPK is not affected, but its activation contributes to and is required for apoptosis. In conclusion, RB may play a role in an alternative pathway to cell death when cells fail to activate caspases in response to death stimuli such as γ-irradiation. In light of the antiapoptotic effect that RB had on DU-145 cells exposed to UV irradiation, death pathways mediated by RB may be specific for signaling downstream from double-strand DNA breaks induced by γ-irradiation.

RB is a key regulator of the cell cycle that binds to a number of proteins involved in cell-cycle signaling, apoptosis, differentiation, and transcriptional activation (84). RB plays an important role in G1 (85) and S phase (24) cell-cycle arrest after normal cells are exposed to ionizing radiation. Moreover, RB can influence the choice between cell-cycle arrest, allowing for DNA after exposure to ionizing radiation, and apoptosis (86). RB is a tumor-suppressor gene whose expression is lost in a wide range of tumors. Restoration of RB expression to tumor cells that have lost RB expression most commonly provides a protective effect against cell death induction by DNA-damaging agents (32, 33, 87, 88). Loss of RB has been shown to predispose to abnormalities of cell-cycle control and cell death (28). The RB protein itself is a target for caspase cleavage during apoptosis (49–54). Mutation of the caspase cleavage sites in RB protein diminishes the death response and results in hyperproliferation of neuronal cells in genetically altered mice (26, 27, 86). It was therefore quite unexpected to find that DU-145 prostate cancer cells, which have a very poor apoptosis response to ionizing radiation, were sensitized to apoptosis by restoration of wild-type RB gene expression (39). Clearly in this aggressive cancer cell line with mutant P53 (89), the expression of RB had proapoptotic effect after γ-irradiation and had minimal effect on cell-cycle arrest (39).

Apoptosis in DU-145 cells expressing RB may have been potentiated by the absence of caspase activation in these cells. Caspase activation may have cleaved RB protein and thereby attenuated DU-145 cell death signaling. Although the caspase cleavage pathways are intact and can be activated by agents
such as okadaic acid, γ-irradiation resulted in no detectable caspase activation in DU-145 cells (39). We did observe cytochrome c egress in irradiated B5 and DU-2.16. Cytochrome c egress from mitochondria accompanies activation of the proapoptotic Omi/HtrA2 serine protease (90, 91). We previously showed that the serine protease inhibitor TLCK, but not the caspase inhibitor zVAD, blocked RB-mediated DU-145 cell death, consistent with dependence of DU-145 cell death on Omi/HtrA2 (39).

The experiments in this paper examined downstream targets of RB cell death signaling. One likely mediator of apoptosis is ABL because it is known to be inhibited by RB binding and known to mediate apoptosis when it is localized to the nucleus (13, 74). Our data are consistent with an important role for ABL in RB-mediated cell death. However, it appeared that whereas ABL was downstream from RB, ABL was not activated directly by interaction with RB since RB variant proteins that had no ABL binding-mediated cell death, but those with constitutive ABL binding blocked the cell death response. Second, we observed a biphasic activation of ABL kinase activity in irradiation DU-145 cells expressing RB, whereas ABL kinase activation in native DU-145 cells occurred only early after irradiation. Thus it appeared that RB mediated a late and secondary activation of ABL kinase, as diagrammed in Fig. 8.

In the presence of RB expression, 20 Gy of irradiation induced activation of JNK within 8 h. Using JUN phosphorylation site mutants, we also showed that JUN phosphorylation was important for RB-mediated apoptosis as was transactivation, perhaps mediated by the leucine zipper protein interaction domain. A JUN DNA binding mutant did not block RB-mediated cell death. JNK activation preceded the peak of ABL kinase activation. It is possible that JNK activation was responsible for ABL kinase activation by inducing interaction of JUN and ABL. JUN and ABL have been shown to interact directly in a circuit of phosphorylation that also involves JNK (22). Although it is possible that JNK mediates activation of ABL kinase by RB, we do not know how RB causes activation of JNK. In cells in which RB is antiapoptotic, JNK is downregulated, perhaps by direct interaction with the N-terminal domain of RB (19, 92). Whether under other circumstances RB interaction with JNK can activate the kinase activity remains to be shown.

p38 MAPK activation has been shown to inhibit RB repression of E2F1 activation (47). The robust activation of p38 MAPK activity may be the reason we did not observe any activation of E2F1 transcriptional activity after irradiation of DU-lux or RB-2.16 cells (Fig. 1E). It is noteworthy that expression of exogenous RB in DU-145 cells caused only minor alteration in cell growth and did not appreciably alter the cell cycle, consistent with a negligible effect of RB expression on E2F1 activity in these cells (39).

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