Role for E2F in DNA Damage-induced Entry of Cells into S Phase

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Abstract

Mammalian cells respond to ionizing radiation (IR) with transient cell cycle arrest and induction of apoptosis. Here we show that IR increases the expression of the E2F-1 transcription factor and the entry of cells into S phase. E2F-1 transactivation function is inhibited by cyclin A-kinase to ensure orderly progression through S phase. However, in contrast to proliferating cells, IR treatment results in down-regulation of cyclin A-kinase. Expression of a dominant negative form of the E2F heterodimeric partner DP-1 confirmed the involvement of E2F in IR-induced S-phase entry. These findings also support opposing signals involving the induction of E2F and the down-regulation of cyclin A-kinase in the IR response.

Introduction

The cellular response to IR includes transient cell cycle arrest, activation of DNA repair, and, in the event of irreparable damage, induction of apoptosis. Cells also respond to IR with the induction of early response genes (1-4). The findings that IR induces c-jun gene expression (1-3) and that Jun/AP-1 is involved in G_0/G_1 to S-phase progression (5, 6) provided support for an IR response involving entry into S phase. IR also induces transcription of the EGR-1 early response gene that encodes a protein implicated in mitogenesis (2, 4, 7). Indeed, other studies have shown that after a transient delay in G_1, irradiated cells exhibit a reduction in the percentage of G_1 cells that is temporally and functionally associated with the activation of early response genes (8, 9). Moreover, this entry into S phase is temporally related to the induction of apoptosis in certain cells (10).

The E2F family of transcription factors (E2F-1 through E2F-5, DP-1, and DP-2) regulates the expression of genes whose products control the entry into and execution of S phase (11). The transcriptional activity of E2F-1 is repressed by the dephosphorylated form of Rb (12). Phosphorylation of Rb by cyclin-dependent kinases before entry into S phase disrupts the interaction between Rb and E2F-1 (12) and renders E2F-1 capable of activating gene expression (12). The E2F proteins form active complexes as heterodimers with DP-1 or DP-2 (13-15). DP-1 mutants that retain E2F binding but not DNA binding arrest cells in G_1 (16). Moreover, overexpression of E2F induces entry into S phase and DNA synthesis (17-20). Overexpression of E2F-1 also leads to apoptosis by p53-dependent mechanisms (18, 19, 21). The finding that E2F-1-/- mice exhibit defects in apoptosis and aberrant cell proliferation has suggested that E2F-1 functions to regulate apoptosis and suppress growth (22).

The present studies demonstrate that the cellular response to IR involves two conflicting signals, induction of E2F-1 and down-regulation of cyclin A-kinase activity.

Materials and Methods

Cell Culture and Cell Cycle Analysis. Human HL-60 cells were grown in RPMI 1640 containing 10% heat-inactivated fetal bovine serum, 1 mm l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. The null pCMV-HA vector and pCMV-HA-DP-1 ΔC1-410 deletion mutant were introduced into HL-60 cells by electroporation. Stable lines were established by selection in G418. Irradiation was performed at room temperature in a Gamacell 1000 (Atomic Energy of Canada, Ottawa, Canada) with a 137Cs source emitting a fixed dose of 13.3 Gy/min. DNA content was assessed by staining ethanol-fixed cells with propidium iodide and monitoring by FACScan. Cell cycle distribution was determined with a ModFit LT program (Verity Software House, Inc.). Cells were labeled with [3H]thymidine (DuPont New England Nuclear) for 4 h after exposure to IR.

Immunoblot Analysis. Cells were lysed in 1% SDS-PAGE, transferred to nitrocellulose filters, and analyzed with anti-E2F-1 (SC-251; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-E2F-2 (SC-633), anti-E2F-3, anti-E2F-4 (SC-866), and anti-PCNA (SC-56). Proteins were detected with an enhanced chemiluminescence system (Amersham, Arlington Heights, IL).

Immunoprecipitates and Kinase Assays. Cell lysates were incubated with anti-cyclin A (SC-239) for 2 h at 4°C and then for 60 min with protein A-Sepharose. Immunoprecipitates were analyzed by histone H1 kinase assays (23) or immunoblotting with anti-cyclin A, anti-Cdk2 (SC-163), or anti-Cdc2 (SC-54).

DNA Fragmentation Analysis. DNA was isolated as described and analyzed in 2% agarose gels (10).

Results and Discussion

Flow cytometry was performed on IR-treated cells to assess the relationship between cell cycle distribution and the induction of apoptosis. Exposure to 10 Gy of IR resulted in the time-dependent appearance of cells with sub-G_1 DNA content (Fig. 1A) and intercromosomal DNA fragmentation (data not shown). IR exposure was also associated with an increase in S-phase cells that corresponded temporally with an induction of sub-G_1 DNA populations (Fig. 1A). The accumulation of cells with both increased sub-G_1 and S-phase DNA content was also dose dependent (Fig. 1B). An increase in S-phase cells may be the result of S-phase arrest or increased DNA synthesis. Studies of [3H]thymidine incorporation demonstrated that the IR-induced accumulation of S-phase cells is due to an increase in DNA synthesis (Fig. 1C). These findings suggested that the IR-treated cells exhibit increased entry into S phase and induction of apoptosis.

Whereas overexpression of E2F-1 induces entry into S phase (17-20), we asked whether IR affects E2F-1 levels. The results demonstrate an increase in E2F-1 protein that was detectable at 3 h after exposure to 10 Gy and more apparent at 6-24 h (Fig. 2A). Other studies have demonstrated that overexpression of E2F-4 can drive cell cycle progression (24). However, IR had no detectable effect on E2F-4 levels (Fig. 2B). E2F-2 and E2F-3 were undetectable in control and irradiated cells (data not shown). As observed for entry into S phase, the induction of E2F-1 was dose dependent (Fig. 2B). To define in part the basis for IR-induced E2F-1 levels, we assessed the effects of IR on E2F-1 mRNA levels and E2F-1 protein stability. Northern analysis (1) demonstrated IR-induced increases in E2F-1 transcripts, whereas there was no effect on actin mRNA levels (data not shown).
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A. DNA Content

B. Cell Number

DNA Content

Fig. 1. IR-induced apoptosis correlates with the entry of cells into S phase. A, HL-60 cells were exposed to 10 Gy of IR. At the indicated times, cells were fixed with 70% ethanol, stained with propidium iodide, and subjected to fluorescence-activated cell-sorting analysis. B, cell cycle analysis was performed 7 h after exposure to the indicated doses of IR (cGy). C, cells were exposed to the indicated IR doses. After 3 h, the cells were incubated with 1 μCi/well [3H]thymidine for 4 h at 37°C. The cells were harvested, washed, and monitored for tritium incorporation.

C.

Fig. 2. Cellular E2F-1 levels are induced by IR in a time- and dose-dependent manner. A, cell lysates prepared at the indicated times after exposure to 10 Gy of IR were subjected to immunoprecipitation with anti-cyclin A. The preciptates were assayed for histone H1 phosphorylation (upper panel) and subjected to immunoblotting with anti-cyclin A, anti-Cdk2, or anti-Cdc2.

B.

Fig. 3. IR induces down-regulation of cyclin A-kinase activity. Lysates from control and IR (10 Gy)-treated cells were subjected to immunoprecipitation with anti-cyclin A. The precipitates were assayed for histone H1 phosphorylation (upper panel) and subjected to immunoblotting with anti-cyclin A, anti-Cdk2, or anti-Cdc2.

C.

Fig. 2. Cellular E2F-1 levels are induced by IR in a time- and dose-dependent manner. A, cell lysates prepared at the indicated times after exposure to 10 Gy of IR were subjected to immunoblot analysis with anti-E2F-1 and anti-PCNA. B, lysates prepared from cells harvested 7 h after exposure to the indicated doses of IR were subjected to immunoblot analysis with anti-E2F-1, anti-E2F-4, and anti-PCNA. C, control and IR (10 Gy)-treated cells were pulse-labeled for 30 min with 100 μCi/ml [35S]methionine-cysteine mix. The cells were chased in medium supplemented with a 10-fold excess of cold methionine for the indicated times. Lysates were subjected to immunoprecipitation with anti-E2F-1. The precipitates were monitored by SDS-PAGE and autoradiography. No significant difference in half-life of the E2F-1 protein was observed after IR treatment in three separate experiments.

C.

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Pulse-chase labeling studies demonstrated a half-life for E2F-1 protein that was similar in control and IR-treated cells (Fig. 2C). These findings indicated that IR decreases E2F-1 protein by inducing expression at the mRNA level.

Cyclin A-kinase, an enzyme required for S-phase progression (25, 26), forms a complex with E2F-1 and negatively regulates DP-1-dependent binding to DNA in S phase/G2 by phosphorylation of DP-1 (27–30). Disruption of the cyclin A-kinase-E2F-1 interaction during S phase is associated with the induction of apoptosis (31). Whereas cyclin A-kinase activity increases during S phase after release of growth-arrested cells by serum addition (29), the effects of IR on this activity are unclear. To address the effects of DNA damage, lysates from IR-treated cells were subjected to immunoprecipitation with anti-cyclin A. Analysis of the lysates from cells stably transfected with pCMV-HA or pCMV-HA-DP-12741° were subjected to immunoblot analysis with anti-HA. B, the pCMV-HA and pCMV-HA-DP-12741° transfectants were exposed to 10 Gy of IR and harvested at the indicated times. Cell lysates were subjected to immunoblotting with anti-E2F-1 (upper panel). Cell cycle analysis was performed on transfectants expressing pCMV-HA or pCMV-HA-DP-12741° at the indicated times after exposure to 10 Gy of IR (lower panel). C, DNA was isolated at the indicated times and monitored for fragmentation by electrophoresis in 2% agarose gels.

Fig. 4. Expression of a dominant negative DP-1 mutant blocks IR-induced apoptosis. A, lysates from cells stably transfected with pCMV-HA or pCMV-HA-DP-12741° were subjected to immunoblot analysis with anti-HA. B, the pCMV-HA and pCMV-HA-DP-12741° transfectants were exposed to 10 Gy of IR and harvested at the indicated times. Cell lysates were subjected to immunoblotting with anti-E2F-1 (upper panel). Cell cycle analysis was performed on transfectants expressing pCMV-HA or pCMV-HA-DP-12741° at the indicated times after exposure to 10 Gy of IR (lower panel). C, DNA was isolated at the indicated times and monitored for fragmentation by electrophoresis in 2% agarose gels.

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A.

B.

C.

References


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