Apo2 Ligand/TNF-related Apoptosis-inducing Ligand and Death Receptor 5 Mediate the Apoptotic Signaling Induced by Ionizing Radiation in Leukemic Cells

Bendi Gong and Alex Almasan

Department of Cancer Biology, Lerner Research Institute, and Department of Radiation Oncology, The Cleveland Clinic Foundation, Cleveland, Ohio 44195

ABSTRACT

Ionizing radiation is a major tool for cancer treatment. The response of eukaryotic cells to ionizing radiation includes apoptosis, a process which requires activation of multiple genes. We sought to determine whether radiation-induced gene expression plays a role in radiation-induced apoptosis. We found Apo2 ligand (Apo2L, also called TRAIL) mRNA induction following γ-irradiation of Jurkat, MOLT-4, CEM, and PBMC, all human T lineage-derived cells. Increased Apo2L protein levels were found in MOLT-4 and Jurkat cells. Radiation also activated the Apo2L death receptor (DR5) (also called Apo2, TRAIL-R2, or KILLER) in MOLT-4 cells, which harbor a wild-type p53. We isolated 1152 bp of 5′ flanking region of the Apo2L gene and a shorter fragment of 716 bp, both of which showed promoter activity driving the expression of a luciferase reporter gene; however, the response to radiation in MOLT-4 cells was lost when only 430 bp of 5′ proximal flanking sequence was maintained. Exogenous Apo2L induced phosphorylation of serine on cell membranes, caspase 8 and caspase 3 activation, key markers of apoptosis, confirming that the Apo2L/DR5 pathway is functional in these cells. Bid, a Bcl-2 family protein known to contribute to receptor-mediated apoptosis, was also activated. To determine whether Apo2L and DR5 were critical for radiation signaling to apoptosis, we stably expressed a dominant negative DR5Δ receptor in Jurkat cells. Cell survival was significantly augmented, indicating that increased Apo2L expression contributed to radiation-induced apoptosis. Clonogenic assays demonstrated that purified, recombinant soluble Apo2L enhanced the lethality of low, therapeutic doses (1–2 Gy) of γ-irradiation. These data suggest that production of Apo2L may cooperate synergistically with the cytotoxic effect of radiation, and that combinations of Apo2L and radiation may become a powerful tool in clinical therapy.

INTRODUCTION

The response of eukaryotic cells to ionizing radiation includes cell cycle arrest and apoptosis, processes which require activation of multiple genes. We have previously reported that expression of Bax, a death agonist activated by the p53 tumor suppressor protein, was an important effector of radiation-mediated cell death of leukemic cells (1). However, inhibitors capable of preventing radiation-triggered Bax up-regulation were not able to completely block cell death, suggesting that factors other than Bax were also involved in radiation-induced cell death.

An alternative pathway to apoptosis could be mediated by surface receptor signaling, which depends on ligand-receptor interactions for cell death effector activity. These cell surface receptors are part of the TNF receptor family and include Fas (also called CD95/Apo1), DR4 (also called TRAIL-R1; Ref. 2), and DR5 (also called Apo2/TRAIL-R2/TRICK2/KILLER; Refs. 3–6) molecules (7). Engagement of these receptors by the FasL (also Apo1L/CD95L), or the apoptosis-inducing ligand Apo2L (also TRAIL; Refs. 8 and 9) can lead to cell death. Expression of Apo2L at mRNA (8) and protein levels (10) was detected in many human tissues, particularly in those of hematopoietic origin. The receptors for Apo2L, DR4, and DR5 contain a cytoplasmic “death domain” capable of engaging the cell suicide apparatus through an adaptor molecule intermediate such as a Fas-associated death domain protein (11). Most importantly, it was reported that whereas many human tumor cell lines were sensitive to cell-surface or soluble Apo2L, normal cells were not. This apparent protection of normal cells from the cytotoxic effect of Apo2L is believed to be based on a unique set of DcRs; these cells either lack the DcR1 (also TRAIL-R3/LIT; Refs. 3–5, and 12) or have a truncated DcR2 (also TRAIL-R4/TRUNND; Refs. 13 and 14) so they are unable to signal, but compete instead for receptor-binding to Apo2L. An alternative view is that levels of an intracellular inhibitor of caspases, a FLICE-inhibitory protein (15), may provide resistance in normal cells (16). The activation of this receptor-mediated cell death pathway during cancer therapy is still not well understood.

The present experiments were designed to examine the role of Apo2L and DR5 in γ-irradiation-triggered apoptosis. We present evidence that radiation induces both Apo2L and DR5, with Apo2L induction being detected only in T lineage-derived cells. Apo2L regulation is mediated through 5′ flanking sequences we have isolated and characterized. Purified Apo2L induces caspase-dependent apoptosis, and when combined with low doses of radiation, it significantly enhances cell death, indicating that combinations of Apo2L and therapeutic radiation may be useful in clinical cancer therapy.

MATERIALS AND METHODS

Cell Culture and Treatments. Human acute T lymphoblastic leukemia MOLT-4 and CEM-CM3, and the T-cell leukemia Jurkat were grown in RPMI 1640. A549 lung carcinoma cells were grown in DMEM. All media contained 10% (vol/vol) heat-inactivated FCS, 50 units/ml penicillin, and 50 mg/ml streptomycin (Life Technologies, Inc., Gaithersburg, MD). All cells were obtained from the American Type Culture Collection (Rockville, MD). Exponentially growing cells were adjusted to a density of 1–2 × 10⁶ cells/ml the day before the experiment was performed. Human PBMCs were isolated by centrifugation over Ficoll-Hypaque density gradient sedimentation (Amersham-Pharmacia Biotech, Piscataway, NJ) from blood samples obtained from healthy adult donors. Jurkat cells were transfected with pcDNA3-DR5Δ (residues 1 to 268), as described (17). DR5Δ lacks the death domain, and has been shown to function as a dominant-negative inactivating the function of the endogenous DR5 (4). DR5Δ also contains a FLAG epitope-tag which facilitates examination of its expression levels. Transfected cells were selected in the presence of 1 mg/ml G418 (Life Technologies, Inc.) containing medium and subsequently maintained with 0.5 mg/ml of G418.

Irradiation was performed as described previously (1) with a 137Cs source emitting at a fixed-dose rate of 2.8 Gy/min. The Fas response was examined with acetyl-Ile-Glu-Thr-Asp-pNA; DEVD, acetyl-Asp-Glu-Val-Asp-pNA; Gy, gray; FACS, fluorescence-activated cell sorter; TRAIL, TNF-related apoptosis-inducing ligand.
using the Fas agonistic mAb (clone CH11; Panvera, Madison, WI) and Fas-Fc with enhancer (Alexis Corporation, San Diego, CA). All chemicals, unless specified, were obtained from Sigma Chemical Co. (St. Louis, MO).

RNA Analyses. Total RNA was isolated from cells at various intervals post-irradiation using the Trizol reagent (Life Technologies, Inc.). To determine the steady-state levels of RNA, we used the RiboQuant system (PharMingen, San Diego, CA) for RNase protection assay with multiprobe template sets which allow simultaneous quantitation and characterization of multiple RNA molecules, as described (1). The hAPO-3 and hAPO-3C template sets (PharMingen) were used for the T7 polymerase-directed synthesis of high specific activity [32P]-labeled antisense RNA probes. The hAPO-3 probe set contains the following genes: (a) the receptors DR3 and TNFRp55; (b) the ligands FasL and Apo2L; (c) the adapters FLICE, Fas-associated death domain; (d) RIP (receptor-interacting protein, which interacts with Fas), as well as FAP (Fas-associated protein) and FAP (PNP1 protein tyrosine phosphatase 1E). hAPO-3C contains the DR4, DR5, and DcR1 receptors for Apo2L. Both sets contain the housekeeping gene L32 as an internal control.

To amplify DcR2, the sense 5'-catgagcttcgggaccgac-3' and antisense 5'-gatagggagaggcaagtggg-3' primers were used as well as those for GADPH with RNA isolated as above. The RT-PCR reaction was carried out as described (1).

Promoter Activity. By searching the National Center for Biotechnology Information using the Basic Local Alignment Search Tool, we found that the Homo sapiens chromosome 3, clone hRPK4_A_1A (accession no. AC007051), contained the whole Apo2L/TRAIL gene (five-exon structure) as well as the 5' upstream promoter region. The primers for the ApoP, ApoP/ APO2L/TRAIL information using the Basic Local Alignment Search Tool, we found that the Homo sapiens chromosome 3, clone hRPK4_A_1A (accession no. AC007051), contained the whole Apo2L/TRAIL gene (five-exon structure) as well as the 5' upstream promoter region. The primers for the ApoP, ApoP/1152, 5'-gctcggagactttggggacaaa-3', ApoP176, 5'-gctcggagctgcagcctcaacacc-3', ApoP430, 5'-gctcggagctggcagcagc-3' and ApoP/anti- sense, 5'-cgaagcttcatgctgccacagtgcaaccac-3' were used to generate, by PCR, the fragments of the ApoP. The PCR reaction was done using the Expand High Fidelity PCR System (Roche Molecular Biochemicals, Indianapolis, IN) following the manufacturer's protocol. MOLT-4 genomic DNA, isolated by QIAamp Tissue Kit (QIAGEN, Valencia, CA), was used as the template. The amplified 1152-, 716-, and 430-bp DNA fragments were subcloned into the pGL2 plasmid containing the luciferase reporter gene (Promega, Madison, WI), and their nucleotide sequence was confirmed by DNA sequence analysis, as described (1).

The human ApoP activity was determined in MOLT-4 cells, as described (17), after cotransfection with 1 μg of Apo2L luciferase reporter and 0.05 μg of pCMV-LacZ plasmid DNA. At 32 h after transfection with the DMRIE-C reagent (Life Technologies, Inc.), the cells were irradiated, and 4 h later, luciferase activity was determined and normalized to values of β-gal. Each assay was performed in triplicate.

Apoptosis, Cytotoxicity, and Clonogenic Assays. Phosphatidylserine exposure on cell membranes was determined with FITC-Annex V by flow cytometry as described (1, 18) using a FACScan and analyzed with CellQuest software (Becton Dickinson, San Jose, CA) on mean values obtained from the cell population from which debris were gated out. Caspase activity was measured as described (1), using the IETD- and DEVD-pNA-derived chromogenic substrates for caspase 8 and -3 activity, by enzyme-catalyzed release of pNA monitored at 405 nm.

γ-irradiation and recombinant soluble Apo2L-treated cells were seeded (2–3 × 10^4 cells) in 96-well plates. Cell viability was examined 24 h later with the CellTiter 96 Aqueous One Solution Reagent (MTS; Promega) to measure tetrazolium reduction, as indicated by absorbance at 490 nm using an ELISA reader (Spectramax 340). All determinations were done in triplicate.

For clonogenic assays, recombinant soluble Apo2L was added 16 h before γ-irradiation to U-bottomed 96-well plates with each well containing 4 μl of medium. Cell colonies were counted 14 days later. Cellular clonogenicity was calculated by the formula: % Clonogenicity = (ln [96/Neg Wells]) (plate density) × 100, where Neg Wells represent the number of wells that have failed to grow to 50 cells (19). MOLT-4 and Jurkat cells have a cloning efficiency of 40% and 66.5%, respectively. Ps were determined by the t test using Microsoft Excel.

Preparation of Recombinant Soluble Apo2L/TRAIL. Escherichia coli BL21 strain was transformed with a His-tagged pET28b-TRAIL (residues 95 to 281) plasmid (22) given generously from Drs. E. S. Almohr and M. S. Stavivakou (4). Expression of the protein was induced with 0.4 mM isopropyl-1-thio-β-D-galactopyranoside for 2 h, and bacteria were pelleted and sonicated in a buffer containing 50 mM Tris-HCl, (pH 8.0), 2 mM EDTA, 0.1% Triton X, and 100 μg/ml lysozyme. The supernatant was collected after centrifugation at 14,000 × g for 15 min and used for additional purification. The recombinant soluble Apo2L (used in Fig. 6) was then affinity-purified by His6-resin affinity purification following the manufacturer's instructions (Novagen, Milwaukee, WI). Apo2L protein specificity was confirmed by SDS-PAGE and Western blot analysis with an anti-Apo2L antibody (C9, Santa Cruz Biotechnology, Santa Cruz, CA). For some experiments (Figs. 4 and 5) a commercial rhApo2L was used, containing the extracellular domain of Apo2L fused at the NH2 terminus to a FLAG-tag and an 8-aminoo acid linker peptide. A combination of Apo2L protein and enhancer have been used according to manufacturer’s specifications (Alexis Corporation, San Diego, CA).

Immunoblotting and Antibodies. Cell lysates were resolved by one-dimensional SDS-PAGE under reducing conditions, as described (1). The blots were then incubated with primary antibodies from PharMingen (Apo2L, 2 μg/ml), Sigma (FLAG M5, 1:1000 dilution; β-actin, 1:5000 dilution), Santa Cruz (caspase 3 p20; 1:500 dilution; caspase-8 H277; 1:500 dilution), or from X. Wang (University of Texas Southwestern (20); Bid polyclonal, 1:1000 dilution) in Tris-buffered saline with 0.05% Tween 20 containing 5% nonfat dry milk for 16 h at 4°C. The blots were then washed in Tris-buffered saline with 0.05% Tween 20, followed by incubation with the secondary antibody conjugated to horseradish peroxidase, and developed using the enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL) followed by exposure to X-ray film (Eastman Kodak).

RESULTS

Ionizing Radiation Induces Apo2L in T Lineage-derived Cells. We sought to examine whether radiation affected expression of cell DRs, their specific ligands and interacting partners, known to be involved in cell-surface receptor-mediated apoptosis (7). The mRNA expression of PBMC and Jurkat, representing normal and tumor T cell-derived cells, were examined using a multiprobe RNase protection assay. Jurkat cells were chosen because they do not have functional p53 and, therefore, radiation does not activate p53-target genes known to be induced during apoptosis, such as Bax. Most significantly, we found a 1.8-fold up-regulation of Apo2L at 4 h following 2 Gy of γ-irradiation in PBMCs. Moreover, Apo2L expression was also induced two-fold at 4 h post-irradiation in Jurkat cells (Fig. 1). Similar results were obtained with another T cell-derived tumor cell line, CEM-CM3, but not in several other human tumor cell lines (data not shown). These data indicate that radiation-induced Apo2L expression was observed in both normal as well as T cell-derived tumor cell lines.

In addition to Apo2L, we found that Fas levels were also up-regulated in PBMCs, whereas Fas-L expression remained unchanged. Interestingly, PMBC was the only cell type in which Fas-L levels were high enough to be detected by RNase protection. As expected, Jurkat cells, which lack functional p53, did not up-regulate Fas following irradiation, confirming the reported p53 requirement for Fas up-regulation (21). Taken together, these results indicate that radiation-induced Apo2L expression was T-lineage dependent, but independent of wild-type p53 function.

Activation of the Apo2L/DR5 Pathway by Ionizing Radiation in MOLT-4 Cells. To further characterize Apo2L regulation, we next examined its expression in MOLT-4 cells, which we have previously characterized for the role of p53-dependent bax induction in apoptosis (1). Control and irradiated MOLT-4 cells were examined at 2 to 8 h after γ-irradiation, a time period during which no cytotoxic effects were observed. Most significantly, we found that Apo2L mRNA was induced significantly after 10 Gy of γ-irradiation (Fig. 2A, top). There was no detectable Apo2L expression in untreated MOLT-4 cells or in those examined 8 h post-irradiation, indicating a transient radiation-dependent regulation. Moreover, there was no significant change in the expression levels of most of the other transcripts, including the housekeeping gene L32, indicating a specific change in expression
levels of Apo2L. A time and dose-response experiment indicated a transient increase in Apo2L expression at all radiation doses tested (2–10 Gy; Fig. 2B). However, the magnitude of this response, a 2.2- to 3-fold increase at the lower 2–4 Gy dose, was considerably less than the 8.5-fold increase obtained after 10 Gy. Similarly, Fas was induced up to eight-fold at doses as low as 2 Gy (at 8 h following irradiation) compared with untreated cells. Treatment with 4 or 10 Gy γ-irradiation resulted in higher induction levels with a more substantial increase at earlier (4.5- versus 2.5-fold at 2 h) compared with later times (8 to 10.5-fold at 8 h; Fig. 2B). These results indicate a time and dose-dependent induction of both Apo2L and Fas, but with a different kinetics of induction of the two genes.

We next sought to examine the expression and regulation of the receptors that mediate Apo2L signaling. We found that only DR5 was expressed in MOLT-4 and Jurkat cells (Fig. 2A, bottom), with no detectable DR4 and DcR1 present (data not shown). Following irradiation, DR5 levels were increased in MOLT-4 cells, which harbor wild-type p53, but not in Jurkat cells, which have a p53 mutation. Because Apo2L was reported to also signal through DcR2, we examined its expression by RT-PCR. There was no DcR2 expression in either MOLT-4 or Jurkat cells, either before or after radiation treatment. In contrast, under the same experimental conditions, DcR2 was readily detectable in A549 lung carcinoma cells (Fig. 2C), as reported previously (13), whereas the levels of GAPDH, an internal control, were unchanged.

Immunoblot analyses, using anti-Apo2L antibodies, showed that Apo2L protein levels were also up-regulated 2- to 3.5-fold in irradiated Jurkat and MOLT-4 cells, respectively, a pattern of induction consistent with that detected for RNA levels. This increase was specific to Apo2L, as there were no changes in the levels of β-actin (Fig. 2D).

To determine the effect of promoter sequences on Apo2L regulation, we isolated 1152 bp of 5′-flanking Apo2L DNA sequence upstream of the Apo2L translation initiation site and created two deletion promoter constructs: ApoP/716 and ApoP/430. Each construct was used to transfect MOLT-4 cells, which were then either left untreated or subjected to 10 Gy of γ-irradiation. Compared with control, the expression of the luciferase gene driven by ApoP/1152 was induced up to 2.3-fold by irradiation (Fig. 3). Similarly, ApoP/716 showed a substantial, up to 1.8-fold, increase in promoter activity in irradiated cells. In contrast, there was no significant change in the expression of the luciferase gene driven by ApoP/430 under similar conditions. These data suggest that the 5′-flanking Apo2L DNA sequence contained between nucleotides −1152 and −430 is required for radiation-induced ApoP activity.

Radiation-induced Apoptosis Is Dependent on Apo2L/DR5 Function. Apo2L binds to several receptors, of which only DR5 is expressed in Jurkat and MOLT-4 cells (Fig. 2). To examine the extent to which Apo2L expression contributes to cell death, we stably expressed a FLAG-tagged dominant-negative DR5Δ into Jurkat cells; these cells were chosen because no other known death effectors are induced. In contrast, multiple death effectors are up-regulated following irradiation of MOLT-4 cells, such as Bax (1), and therefore it would be difficult to dissociate the effect of Apo2L from that of other death-promoting proteins.

If Apo2L expression had a role in apoptosis, then blocking the Apo2L signaling pathway should have an impact on cell survival. Radiation treatment induces phosphatidylinerse exposure on the cell membrane, a relatively early marker of apoptosis (1, 18). We found that 27% of Jurkat cells were viable, as they did not stain with Annexin V (which binds to phosphatidylinerse), 24 h after 20 Gy of γ-irradiation. In contrast, 56% of Jurkat/DR5Δ cells survived (Fig. 4A). Moreover, there was a marked difference in survival between parental and DR5Δ-expressing Jurkat cells as early as 12 h following irradiation (Fig. 4B). Thus 67, or 54%, of Jurkat/DR5Δ cells survived, compared with 50, or 31%, of irradiated parental cells examined at 12 or 24 h, respectively. These cells expressed the DR5Δ receptor, as shown by the presence of the FLAG epitope with which they were tagged (inset). Fas-Fc pretreatment had no significant effect on survival of DR5Δ/Jurkat cells and only a slight effect on survival of Jurkat cells 12 h after treatment. Furthermore, the Jurkat/DR5Δ cells were resistant to the cytotoxic effect of Apo2L, but remained sensitive to killing by an anti-Fas agonistic antibody, indicating the specificity of DR5Δ function (Fig. 4C). Fas-Fc pretreatment had no significant effect on survival of Apo2L-treated DR5Δ cells, but prevented, as expected, the cell death of Fas-treated cells. These results indicate that blocking the DR5-mediated Apo2L signaling has a significant impact on cell survival and that γ-irradiation-induced Apo2L expression contributes to the cell death of hematopoietic cells.

Apo2L Induces Apoptosis through Activation of Caspase 8, Bid, and Caspase 3. Apo2L can induce apoptosis in a variety of tumor cells. However, the mechanism of Apo2L-induced apoptosis remains to be clarified. Apo2L induced rapid apoptosis of Jurkat cells in a time-dependent manner. Cells started to lose viability as early as 4 h after treatment, with most of cells being Annexin V-FITC-positive by 12 h (Fig. 5A). To assess whether caspases were activated in our system, we determined caspase activity in Apo2L-treated cells. Apo2L induced effectively IETD- and DEVD-pNA cleavage activity, corresponding to caspase 8 and caspase 3; this activity started at 2 h and reached a maximum at 4–6 h (Fig. 5B). Moreover, Western blot analyses show that the procaspase 3 was proteolytically cleaved to the
p17 kDa fragment, known to be the activated form of caspase 3. To address the pathway by which caspase 3 was activated, we examined in more detail caspase 8, an apical caspase known to be the first caspase to be activated in receptor-mediated apoptosis. Levels of procaspase 8 were decreased substantially by 4 h, with further time-dependent decrease in protein levels (Fig. 5C). These results, taken together with the increase in IETD-pNA cleavage activity, indicate that caspase 8 is activated by Apo2L. Finally we examined Bid, a Bcl-2 family member also recently reported to be involved in receptor-mediated apoptosis and to further caspase 3 activation (20, 22). Bid levels were significantly reduced upon Apo2L treatment, indicating processing of the full-length Bid to a p15- or p13-kDa fragment not detectable under our experimental conditions. Activation of caspase 8 and caspase 3 indicates a caspase-dependent apoptosis, with Bid most likely contributing to the amplification of the caspase cascade.

Combination of Apo2L and Radiation Treatments Greatly Enhance Lethality. To further examine a direct contribution of Apo2L induction to cell death, we affinity-purified recombinant, soluble Apo2L protein, which was shown by SDS-PAGE to be present as a 28-kDa monomeric protein (Fig. 6A). Apo2L protein specificity was confirmed by Western blotting with an anti-Apo2L and anti-β-actin antibodies. The Apo2L protein levels were quantitated using NIH Image following normalization to β-actin.
Apo2L concentrations. For Jurkat cells, survival was reduced from 35.9% to 29.5% ± 2.6% or 7.3% ± 1.7% (P < 0.01) for the 1- or 2-Gy dose, respectively. When higher concentrations of Apo2L (0.2 µg/ml) were used, survival decreased from 52.2% ± 6.8% to 22.9% ± 4.8% or 6.7% ± 0.9% (P < 0.01) at the two radiation doses. These data indicate that radiation kills lymphocytes by a Fas-independent mechanism (23). Apo2L induction in T cells could be most important because radiation could selectively kill tumor cells, which express only the DR5 and/or DR4 receptors, but not normal cells, which also express DRs or FLICE-inhibitory protein.

To elucidate the mechanism of Apo2L regulation, we isolated and characterized the 5'-flanking region of the human Apo2L gene, which showed promoter activity in irradiated MOLT-4 cells. The consensus sequences for several transcription-regulatory factor-binding motifs were identified to be present in Apo2/P430, but not the Apo2/P430 promoter region, including sites for OCT-1, SP-1, GATA1–3, and C/EBP. These sites, located in the promoter region between nucleotides −1152 to −716 (OCT-1, SP-1, GATA1–3), and −716 to −430 (C/EBP) could mediate the radiation-caused transcriptional induction of Apo2L. In fact, a DNA-binding activity was reported in several tumor cell lines following irradiation for both OCT-1 and SP-1 (24). The exact role of these elements for the radiation response needs to be investigated further.

A functional role for Fas in the radiation response has been reported in T cells from animals with defective expression of Fas or FasL
function, which showed reduced sensitivity to radiation-induced apoptosis (25). Fas was induced in our system too, consistent with its up-regulation, in a p53-dependent manner, following treatment with DNA-damaging and oxidant agents of a number of hematopoietic and solid tumor cell lines (21). However, MOLT-4 cells were resistant to Fas treatment (500 ng/ml for 24 h, data not shown). Moreover, in Jurkat cells, which harbor mutant p53, Fas was not induced by radiation, there was no detectable FasL mRNA present, and Fas-Fc pretreatment had no effect on radiation-induced cell death. Most importantly, DR5Δ protected Jurkat cells from radiation and Apo2L-induced, but not Fas-induced, cell death, indicating that the Fas/FasL system is not involved in radiation-induced apoptosis in our system, although it is clearly important for regulation of apoptosis in some other biological systems. This finding is consistent with reports that radiation kills lymphocytes by a Fas-independent mechanism (23) and that chemotherapy-induced apoptosis is not dependent on Fas/FasL interactions (26, 27). In fact, various human lymphoid cell lines differ in their sensitivity toward induction of apoptosis by Apo2L and Fas (28), an observation we have extended to myeloid cells.4

Radiation induces caspase 8 and -3 activation in Jurkat (data not shown) and MOLT-4 cells, leading to proteolytic cleavage of cellular proteins such as poly(ADP-ribose)polymerase (1). Treatment of Jurkat cells with purified Apo2L also induced caspase 8 and -3 cleavage and activation, similar to previous reports (28, 29), indicating that radiation may mediate its apoptotic effects by engaging the caspase cascade not only through increased levels of the DR5 receptor, but also by increasing the levels of Apo2L. Bid activation indicates the requirement for caspase amplification in the Apo2L/DR5 apoptotic pathway by a mechanism reported to take place through translocation of activated Bid to mitochondria, facilitating cytochrome c release and activation of caspase 9 and then caspase 3 (20, 22, 30).

The observations that radiation can induce Apo2L and that low doses of radiation can cooperate synergistically with Apo2L in enhancing cell death may have implications for clinical therapy. This is reminiscent of the synergistic or additive cell killing between TNF and radiation reported in several solid tumor cell lines (31). Gene therapy approaches have been proposed based on radiation-responsive promoters driving TNF expression (32), and TNF expression has been shown to sensitize certain radiation-resistant tumors (33). In addition, there are essential differences between the Apo2L-DR4 or DR5 pathway and those using TNF and FasL, which further indicate that Apo2L might be a safer agent. Thus, at least in some systems, it has been shown that Apo2L kills tumor cells and not normal cells because of the presence on the latter of DcRs (3, 5). More recently, the observations of a lack of cytotoxicity of Apo2L toward normal cells and tissues have been extended successfully to mice (34) and nonhuman primates (35). Several chemotherapeutic agents have been also shown to have a synergistic activity with Apo2L (35, 36), indicating that combination therapies using Apo2L are likely to be widely applicable.

The present investigations enhance our understanding of the mechanism of radiation and Apo2L-induced apoptosis. The observed synergy between Apo2L and low, therapeutic doses of radiation can form the basis for developing strategies for pharmacological intervention, with potential for clinical application. In particular, the specificity of Apo2L cytotoxicity for tumor cells and its systemic distribution reaching metastases and the surgical precision with which ionizing radiation can now be delivered in the clinic could constitute a very attractive combination for enhanced clinical response of tumors resistant to radiation therapy.

ACKNOWLEDGMENTS

We thank Drs. E. S. Alnemri and S. M. Srinivasula for the pET28b-TRAIL and pCDNA3-DR5Δ constructs, X. Wang (U. T. Southwestern) for the Bid

---

antibody, and Amy Raber (Cleveland Clinic Flow Cytometry Core) for expert assistance. The Becton Dickinson FACS Vantage Cell Sorter was purchased through a generous gift from the Keck Foundation. We thank Drs. C. Bevins, Q. Chen, R. M. Mackliss, S. Mazumder, G. Chen, and D. Feng for helpful suggestions on the manuscript.

Note Added in Proof

After this work was completed, it was reported that radiation can sensitize breast carcinoma cells both in vitro and in vivo to Apo2L-induced apoptosis. This synergistic effect was p53-dependent and was suggested to be the result of radiation-induced up-regulation of DR5 (37). However, Apo2L is not induced following irradiation of these breast carcinoma cells, indicating that the synergy between radiation and Apo2L might work through different mechanisms in different cell types.

REFERENCES


© 2000 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from cancerres.aacrjournals.org on May 31, 2017.
Apo2 Ligand/TNF-related Apoptosis-inducing Ligand and Death Receptor 5 Mediate the Apoptotic Signaling Induced by Ionizing Radiation in Leukemic Cells

Bendi Gong and Alex Almasan


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/20/5754

Cited articles
This article cites 36 articles, 21 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/20/5754.full#ref-list-1

Citing articles
This article has been cited by 27 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/20/5754.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.