Parathyroid hormone-related protein ameliorates death receptor-mediated apoptosis in lung cancer cells


Parathyroid hormone-related protein ameliorates death receptor-mediated apoptosis in lung cancer cells. Am J Physiol Cell Physiol 285: C1429–C1436, 2003. First published August 13, 2003; 10.1152/ajpcell.00269.2003.—Parathyroid hormone-related protein (PTHrP) is expressed in more advanced, aggressive tumors and may play an active role in cancer progression. This study investigated the effects of PTHrP on apoptosis after UV irradiation, Fas ligation, or staurosporine treatment in BEN human squamous lung carcinoma cells. Cells at 70% confluence were treated for 24 h with 100 nM PTHrP-(1–34), PTHrP-(38–64), PTHrP-(67–86), PTHrP-(107–139), or PTHrP-(140–173) in media with serum, exposed for 30 min to UV-B radiation (0.9 mJ/cm²), and maintained for another 24 h. Caspase-3, caspase-8, and caspase-9 activities increased fivefold. Pretreatment with PTHrP-(1–34) and PTHrP-(140–173) ameliorated apoptosis after UV irradiation, as indicated by reduced caspase activities, increased cell protein, decreased nuclear condensation, and increased clonal survival. Other peptides had no effect on measures of apoptosis. PTHrP-(140–173) also reduced caspase activities after Fas ligation by activating antibody, but neither peptide had effects on caspase-3 or caspase-9 activity after 1 μM staurosporine. These data indicate that PTHrP-(1–34) and PTHrP-(140–173) protect against death receptor-induced apoptosis in BEN lung cancer cells but are ineffective against mitochondrial pathways. PTHrP contributes to lung cancer cell survival in culture and could promote cancer progression in vivo. The mechanism for the protective effect against apoptosis remains to be determined.

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including human lung cancer cells (4, 19), and it inhibits apoptosis in various cells, including growth plate chondrocytes, pancreatic β-cells, LNCaP prostate carcinoma cells, MCF-7 breast carcinoma cells, cerebellar neurons, cytotoxophlasts, and coronary endothelial cells (5, 8, 14, 26, 29). The effects of PTHrP on lung cancer cell apoptosis are unknown.

The first goal of this study was to investigate the effects of PTHrP on the sensitivity of BEN human squamous bronchial lung carcinoma cells to apoptosis induced by UV-B irradiation. UV radiation was chosen as the stimulus, because it caused reproducible degrees of apoptosis in preliminary studies. UV radiation can cause apoptosis through death receptor- or mitochondria-initiated pathways (2, 31), also known as the extrinsic and intrinsic pathways, respectively. Thus studies of UV radiation-induced apoptosis may elucidate mechanisms common to lung cancer cell apoptosis in a variety of settings, including DNA damage after chemotherapy or radiation. We initially measured caspase-3 activity in cells treated with peptides spanning the entire PTHrP sequence, because biological activity has been ascribed to multiple portions of the molecule (11, 13, 23, 24, 30, 34). Two peptides inhibited caspase-3, PTHrP-(1–34) and PTHrP-(140–173), and were evaluated further for effects on caspase-8 activity, caspase-9 activity, and nuclear fragmentation/conden-
sation. Our second goal was to determine whether these peptides had inhibitory actions on extrinsic or intrinsic apoptosis pathways. Caspase activities were assayed after Fas ligation or staurosporine treatment, stimuli that initiate solely death receptor and mitochondrial pathways, respectively. Finally, we examined the effects of PTHrP-(1–34) and PTHrP-(140–173) on clonal survival of BEN cells after UV irradiation.

METHODS

General Protocols

Cell culture. BEN cells (generously provided by T. J. Martin) were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine and incubated at 37°C in a humidified incubator with 5% CO2–95% air. Cells were plated in 100-mm dishes for experiments involving protein measurements or caspase assays and in NUNC Slide-Tek wells for nuclear condensation experiments.

Treatments. Cells were treated with 100 nM PTHrP-(1–34), PTHrP-(38–64), PTHrP-(67–86), PTHrP-(107–138), or PTHrP-(140–173) (Bachem, Torrance, CA) for 24 h before apoptotic stimuli. Peptides were dissolved in growth medium.

Apoptotic stimuli. Cells were exposed to apoptotic stimuli at 60–70% confluency. Three different treatments were employed: UV-B irradiation, ligation of Fas with Jo-2 activating Fas antibody (15 μg/ml; Pharmingen, San Diego, CA) for 7.5 h, or incubation with 1 μM staurosporine for 48 h. Control cells for the staurosporine experiments received an equal volume of the DMSO vehicle (1:1,000). UV-B radiation was administered in a sterile hood at room temperature by a short-wave UV transilluminator (Fotodyne, New Berlin, WI) placed over the cells for 30 min. The lid of the cluster well plate was left in place. The cells were then allowed to recover for 24 h at 37°C in a humidified incubator before they were further processed for apoptotic markers. Total dose of UV radiation was 0.9 J/cm² measured at 310 nm by a UV detection J meter (UVP, Upland, CA). The probe was placed at an equivalent distance from the UV source and under the cluster plate top to mimic the conditions under which the cells were exposed.

Fluorescent microscopy. Fluorescent cell imaging was performed with an Olympus BX60 upright microscope with fluorescent attachments (Olympus America, Melville, NY). Light from a 100-W mercury lamp was passed through an Omega UV-2E/C filter set for Hoechst 33342 fluorescence or an XF-100-2 filter for Alexa 488 fluorescence.

Immunofluorescence. Cells grown on Slide-Tek wells were washed with PBS, fixed with 2% formaldehyde-0.25% glutaraldehyde, and permeabilized with 0.2% Triton X-100 in PBS for 10 min. Non-specific protein binding was blocked in 20% FBS, 0.2% gelatin, and 0.01% azide in PBS. Cells were incubated overnight at 4°C with 20 μg IgM/ml CH11, a primary antibody to Fas (Upstate Cell Signaling, Waltham, MA). On the next day, cells were incubated with a biotinylated anti-mouse IgM followed by streptavidin-Alexa 488 conjugate, washed in PBS, mounted with Prolong Antifade medium (Molecular Probes, Eugene, OR), and examined.

Cell survival assays. A clonal survival assay was used to determine the ultimate effect of PTHrP peptides on cell outcome after UV irradiation. Irradiation was performed as described above, except the dose of UV-B radiation was limited to 60 mJ/cm² delivered over 2 min. After exposure, cells were trypsinized, counted, and stained for viability by trypan blue exclusion. Cells were then replated in fresh medium with 10% FBS at 1,000 viable cells/well in six-well cluster plates. Control cells that were not exposed to UV radiation were also trypsinized and replated at 200 cells/well. PTHrP peptides were present in the medium only during the initial 24-h pretreatment period; i.e., adherence and growth after UV irradiation occurred without exogenous PTHrP peptides. After 12 days, plates were washed and stained with Giemsa stain (Searle Diagnostics, Bucks, UK). Two separate observers, unaware of the experimental group, counted colonies of ≥50 cells. The two results generally agreed within 5% and were averaged for data analysis.

Specific Protocols

Effect of PTHrP peptides on UV radiation-induced apoptosis. Caspase-3 activities were compared among BEN cells treated with 100 nM PTHrP-(1–34), PTHrP-(38–64), PTHrP-(67–86), PTHrP-(107–138), and PTHrP-(140–173) with and without UV irradiation. The subsequent studies focused on PTHrP-(1–34) and PTHrP-(140–173), because these peptides reduced caspase-3 activity in the initial studies, whereas the other peptides did not. Caspase-3 was used as the initial marker for apoptosis, because it is the downstream executioner caspase that catalyzes the cleavage of many key enzymes and structural proteins during apoptosis, such as poly(ADP-ribose) polymerase, PKC-δ, and lamin A (7). Caspase-8 activity, caspase-9 activity, loss of cell mass, and nuclear condensation were quantified as independent measures of cell death and apoptosis.

Effect of PTHrP-(1–34) peptides on death receptor- or mitochondrial-initiated apoptosis. Caspase-3, caspase-8, and caspase-9 activities were investigated after BEN cells were treated with staurosporine to activate the mitochondrial pathway or with activating Fas antibody to initiate the death receptor pathway.
Death receptor activation after UV irradiation. BEN cells were stained for Fas and examined by fluorescent microscopy to investigate whether UV irradiation caused clustering of Fas, an event that indicates activation.

Cell survival after UV irradiation. Clonal survival assays were performed to determine whether the protective effects of PTHrP-(1–34) and PTHrP-(140–173) against apoptosis translated into increased survival.

Measurements

Caspase assays. Adherent cells were washed once in PBS, scraped in 500 μl of caspase lysis buffer [50 mM PIPES-KOH, 2 mM EDTA, 0.1% (wt/vol) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 1 mM dithiothreitol, and 1 μM each leupeptin and pepstatin A], pooled with the nonadherent cells from the same wells, and lysed by sonication. Lysates were kept at −20°C until time of assay. Assays were performed in 96-well plates with protein from cell lysates. The mass of cell protein for each sample was adjusted to 20 μg, measured by the Pierce bicinchoninic acid protein assay. Substrates were acetyl-aspartyl-glutamyl-valyl-aspartyl (DEVD)-7-amino-4-methyl coumarin (AMC) for caspase-3, acetyl-isoleucyl-glutamyl-threonyl-aspartyl (IETD)-AMC for caspase-8, and acetyl-leucyl-glutamyl-histidyl-aspartyl (LEHD)-AMC for caspase-9 (Alexis Biochemicals, Carlsbad, CA). Substrates were diluted to 0.1 mM in caspase assay buffer [50 mM HEPES, 100 mM NaCl, 0.1% (wt/vol) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, and 5 mM dithiothreitol]. Assays were initiated by adding the substrate to the sample well at 4°C and then heating the plates to 37°C. Fluorescence of AMC cleaved from the substrate was detected on a 96-well multilabel reader (Perkin-Elmer, Boston, MA) at excitation and emission wavelengths of 355 and 460 nm, respectively, with a 0.5-s capture window. Fluorescent of AMC cleaved from the substrate was detected on a 96-well multilabel reader (Perkin-Elmer, Boston, MA) at excitation and emission wavelengths of 355 and 460 nm, respectively, with a 0.5-s capture window. Fluorescent readings were taken at 30-min intervals, exported to Microsoft Excel, and converted to relative fluorescent units by subtracting the background fluorescence of the reagent blank for each plate to compensate for plate-to-plate variability. Activities were calculated as the slope of the plot of relative fluorescent unit vs. time over the first 1–2 h, the linear portion of the curve.

Nuclear condensation assessment. Nuclear condensation was quantitated as described previously (13). Adherent cells in Slide-Tek wells were washed with PBS and fixed with 100% methanol. Nuclei were stained for 15 min with 4 mM Hoechst 33342 dye and imaged with a digital camera (SPOT 2e, Diagnostic Instruments, Sterling Heights, MI) at ×200 magnification. Images were saved as 12-bit color TIFF files and converted to 8-bit gray scale for image analysis. Nuclear area was quantitated using NIH Image 1.62. BEN cells that were not exposed to UV radiation or treated with PTHrP were used to establish a lower threshold for the area of nuclei in nonapoptotic cells. Condensed nuclei were defined as those nuclei with area that was less than or equal to the fifth percentile for nuclear areas of normal cells. At least 200 cells were examined for experimental condition and cell isolation, and ≥300 cells were evaluated per control group in establishing the cutoff area.

Data Analysis

Nuclear condensation, caspase activities, cell protein, and cell survival were compared among groups by analysis of variance. Dunnett’s test was used for post hoc pairwise comparisons of individual experimental vs. control values. Significance was accepted when the probability of a type II error was <0.05.

RESULTS

Effect of PTHrP Peptides on UV Radiation-Induced Apoptosis

Effect on caspase activities. On average, UV irradiation caused five- to sixfold increases in caspase-3 activity compared with nonirradiated cells (Fig. 1). Pretreatment with PTHrP-(1–34) or PTHrP-(140–173) slowed the rate of fluorescence release in irradiated cells and attenuated the increase in activity due to UV irradiation by 20–30%. Other PTHrP peptides did not significantly affect caspase-3 activity, and none of the peptides had effects in nonirradiated cells. The effects of PTHrP-(1–34) and PTHrP-(140–173) on caspase-3 activity after UV irradiation were dose dependent (Fig. 2). We also investigated the effects of PTHrP-(1–34) and PTHrP-(140–173) pretreatment on activities of caspase-8 and caspase-9, upstream enzymes in the death receptor- and mitochondria-dependent apoptosis cascades, respectively. UV irradiation increased caspase-8 activity by sevenfold and caspase-9 activity by three- to fourfold (Fig. 3). PTHrP-(1–34) and PTHrP-(140–173) reduced the activities of these caspases after UV irradiation to a similar extent compared with their effects on caspase-3.

![Fig. 1. Effect of parathyroid hormone-related protein (PTHRP) peptides on caspase-3 activity after UV irradiation in BEN cells. UV irradiation caused a 6-fold increase in caspase-3 activity compared with nonirradiated cells (***P < 0.01). Pretreatment with 100 nM PTHrP-(1–34) or PTHrP-(140–173) for 24 h before irradiation reduced caspase-3 activity ~25% (**P < 0.05). Other PTHrP peptides did not have significant effects, and peptides had no effect on nonirradiated cells. Values are means ± SE for 7 separate experiments.](http://ajpcell.physiology.org/)
Effect on loss of cell mass. UV irradiation caused a 50% decline in cell protein compared with nonirradiated cells (Fig. 4). The loss of cell protein was significantly less after treatment with PTHrP-(1–34) or PTHrP-(140–173). Protein in irradiated cells was increased 15% by pretreatment with either peptide (*P < 0.05, n = 6). The effects of PTHrP-(1–34) and PTHrP-(140–173) were not significantly different. The peptides had no effects on total cell protein in nonirradiated cells.

Effects on nuclear condensation. The percentage of cells with condensed or fragmented nuclei increased from 5.0% in controls to 11.3% in UV-irradiated cells (Fig. 5). Treatment with PTHrP-(1–34) and PTHrP-(140–173) reduced nuclear condensation to 5.6% and 5.6% of cells, respectively (*P < 0.05), not significantly different from levels in nonirradiated cells. Figure 6 demonstrates the nuclear morphology of control cells and UV-irradiated cells with and without peptide treatment. Apoptotic bodies and cells with condensed nuclei were present among irradiated cells.
lated cells, but to a lesser degree after PTHrP peptide treatment.

**Effect of PTHrP Peptides on Death Receptor- or Mitochondria-Initiated Apoptosis**

**Fas ligation.** Activating Fas antibody increased caspase-3 and caspase-8 activities in BEN cells by ~70% (Fig. 7). Caspase-9 activity was increased 90 ± 1.5% (P < 0.01; not shown). PTHrP-(140–173) pretreatment reduced caspase activities in Fas-treated cells by one-half, to levels similar to those in control cells without Fas activation.

**Staurosporine treatment.** Treating BEN cells with staurosporine for 48 h increased caspase-3 activity by 75–100% (P < 0.01) but did not augment caspase-8 activity compared with the low activity in untreated cells (data not shown). PTHrP-(1–34) and PTHrP-(140–173) had no effect on caspase-3 or caspase-9 activity after staurosporine.

**Death Receptor Activation After UV Irradiation**

BEN cells underwent immunofluorescent staining to investigate whether UV irradiation caused clustering of Fas receptor. Irradiated cells demonstrated dense

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Fig. 6. Nuclear morphology of BEN cells after UV irradiation and treatment with PTHrP peptides shown in micrographs of nonirradiated (A) and irradiated (B–D) BEN cell nuclei stained with Hoechst 33342. Cells received no PTHrP treatment (A and B) or 24 h of treatment with PTHrP-(1–34) (C) or PTHrP-(140–173) (D) before UV exposure. Cells with condensed and/or fragmented nuclei, indicating apoptosis, are present in all groups after UV exposure but are decreased in cells treated with either PTHrP peptide. Arrowheads, representative apoptotic bodies or condensed nuclei. Micrographs are representative of data shown in Fig. 5.

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Fig. 7. Effect of PTHrP peptides on caspase activity after Fas ligation. BEN cells were treated with 15 μg/ml activating Fas antibody Jo-2 for 7 h. Caspase activities were measured by fluorescent substrate assay as described in Fig. 3 legend. Fas ligation caused significant increases in activities of caspase-3 and caspase-8 (**P < 0.05), as well as caspase-9 (data not shown). Pretreatment with PTHrP-(140–173) significantly reduced activities compared with Jo-2-treated cells without PTHrP supplementation. (**P < 0.05). Peptides had no effect on activities in cells that were not treated with antibody. Values are means ± SE; n = 3.
staining that was primarily localized to the plasma membrane (data not shown). In nonirradiated cells, staining was very faint and diffuse.

**Effects of PTHrP-(1–34) and PTHrP-(140–173) on Cell Survival After UV Irradiation**

UV irradiation for 2 min resulted in a 95% decrease in colony formation after 12 days (Fig. 8). Pretreatment with PTHrP-(1–34) and PTHrP-(140–173) increased colony survival nearly two- and fourfold, respectively, compared with untreated irradiated cells \( P < 0.05 \) and \( P < 0.01 \) vs. no treatment and \( P < 0.05 \) vs. PTHrP-(1–34), \( n = 3 \).

**DISCUSSION**

Our first goal in this study was to investigate the effects of PTHrP peptides on the sensitivity of lung cancer cells to apoptosis. We found that PTHrP-(1–34) and PTHrP-(140–173) protected BEN cells against apoptosis induced by UV-B radiation and Fas ligation. The protection was demonstrated by decreases in activities of caspase-3, caspase-8, and caspase-9 (Figs. 1–3 and 7), reduced nuclear condensation/fragmentation (Figs. 5 and 6), increases in cell mass, and improved cell survival (Fig. 8) after UV irradiation.

Our second goal was to begin to explore the mechanisms for these antiapoptotic effects. Specifically, we wanted to determine the PTHrP effects on extrinsic and intrinsic apoptosis pathways. Because the PTHrP peptides decrease caspase-8 and caspase-9 activity, they could be acting on a death receptor apoptosis pathway alone or on mitochondria-initiated apoptosis. Caspase-8 is activated after recruitment to trimmed death receptors through binding to adapter proteins, such as Fas-associated death domain, and serves as the initiator caspase for the extrinsic pathway (25). Caspase-9 is the upstream caspase in the intrinsic pathway and is activated by binding in complex of apoptotic protease-activating factor-1, cytochrome c released from mitochondria, and dATP (12). We explored this issue by testing the effects of the peptides on caspase activities after stimuli that exclusively work through one or the other pathway. Staurosporine affects apoptosis predominantly through mitochondria, as demonstrated in BEN cells by lack of stimulation of caspase-8. Neither peptide altered caspase-3 or caspase-9 activity after staurosporine treatment, suggesting that they did not act on intrinsic pathways. We tested extrinsic pathways by ligating Fas with an activating antibody, Jo-2. In contrast to the lack of efficacy after staurosporine, PTHrP-(140–173) decreased caspase-8, caspase-9, and caspase-3 activity after Fas ligation (Fig. 7). Thus PTHrP-(140–173) appears to protect BEN cells against apoptosis mediated by the death receptor pathway. Because caspase-8 levels are reduced, the effect appears to be exerted at or upstream of the level of caspase-8. It is not clear why PTHrP-(1–34) differed from PTHrP-(140–173) in effects against Fas-mediated apoptosis. Because both peptides protect against apoptosis after UV irradiation, we expected similar effects vs. Fas antibody. PTHrP-(140–173) could be more potent than PTHrP-(1–34). It exerted greater effects than PTHrP-(1–34) at the same dose in the clonal survival assay (Fig. 8). Alternatively, the lack of effect vs. Fas may reflect differences specific to the stimulus. We found no evidence that PTHrP peptides protect BEN cells against apoptosis mediated solely by mitochondrial mechanisms but have not completely ruled out this possibility.

UV-B irradiation can activate extrinsic and intrinsic apoptosis pathways (2, 31). Our data are consistent with stimulation of the extrinsic pathway in BEN cells. The mechanism may be through aggregation of Fas, as we demonstrated with immunofluorescent staining. Clustering of Fas after UV irradiation is associated with activation of the death receptor pathway in various cell types (2).

PTHrP has been shown to protect against the intrinsic apoptosis pathway in other cells and tissues. In growth plate chondrocytes, PTHrP increases expression of Bcl-2, delaying their maturation and apoptotic cell death (1). MCF-7 breast cancer cells overexpressing wild-type PTHrP were more resistant to apoptosis induced by serum starvation than were cells transfected with vector control and expressed higher Bcl-2-to-Bax and Bcl-X\(_{L}\)-to-Bax ratios (32). Pancreatic islet cells from transgenic mice overexpressing PTHrP under the control of the rat insulin II promoter express three- to fourfold higher levels of Bcl-2 mRNA than islet cells from wild-type mice (5). Finally, increases in PTHrP expression in coronary endothelial cells improve resistance to apoptosis associated with increases...
in Bcl-2 levels (29). To our knowledge, these data are the first demonstration that PTHrP can regulate death receptor apoptosis pathways. These effects were elicited by exogenous peptides and are paracrine in nature. In many cases, PTHrP elicits effects on apoptosis after localization to the nucleus (14, 29, 32), actions that are termed “intracrine” effects. We have not investigated the intracrine effects of PTHrP in lung cancer cells.

To our knowledge, regulatory effects of PTHrP-(140–173) on apoptosis have not been described previously. This region of the PTHrP molecule has been shown to have biological activity in only a few other studies. In cultured articular chondrocytes, PTHrP-(140–173) decreases extracellular pyrophosphate levels (11). A receptor for PTHrP-(140–173) has not been identified. The receptor for PTHrP-(1–34), the combined PTH-PTHrP receptor, is a G protein-coupled receptor and can signal through increases in PKA- or PKC-dependent mechanisms (27). The dose-response experiments (Fig. 2) are evidence that the effects of the peptides on caspase-3 activity are receptor mediated, although other mechanisms have not been excluded. Because the effects of PTHrP-(140–173) on BEN cell apoptosis are similar to the effects of PTHrP-(1–34), PTHrP-(140–173) might act through a G protein-coupled receptor as well. In preconfluent mesenchymal cells, antiapoptotic effects of PTHrP-(1–34) are exerted through increases in cAMP (6). In contrast, PTHrP-(1–34) is proapoptotic in HEK-293 embryonic human kidney cells by acting through a Gα-mediated phospholipase C-Ca2+ signaling pathway (33). In alveolar epithelial cells, protective effects against apoptosis are apparently mediated through a PKC-dependent pathway (13). Further work is necessary to establish the signaling mechanisms for PTHrP-(1–34) and PTHrP-(140–173) in lung cancer cells.

Inhibitory effects on apoptosis would be clinically tenuous if PTHrP simply prolonged the interval between stimulus and lung cancer cell demise or changed the mode of death from apoptosis to necrosis. However, clonal survival studies showed that PTHrP peptides protect the cells from death. The data reflect survival, not increased adherence or proliferation, because the cells were exposed to peptides only through the period of UV irradiation. The overall protection was small, because 87–94% of the cells died after PTHrP treatment, compared with 97% with no treatment. This result is not inconsistent with the effects on caspase activity or nuclear condensation, because the survival outcome would not be expected a priori to change in proportion to changes in these intermediate measures. The high death rate could also represent necrosis or caspase-independent apoptosis occurring along with caspase-mediated apoptosis. Nevertheless, the peptides still increased survival severalfold compared with untreated cells. A small increase in survival could make a large difference in the progression of an exponentially expanding tumor. Thus autocrine protection of PTHrP against apoptosis could contribute to lung cancer survival and increase resistance to chemotherapeutic agents that act through inducing apoptosis.

Further work is indicated to investigate how the antiapoptotic effects of PTHrP-(1–34) and PTHrP-(140–173) interact with the pathophysiology of lung cancer. PTHrP contributes to tumor progression in some animal models of cancer. For example, overexpression of PTHrP in MatLyLu rat prostate carcinoma cells accelerated growth of tumors in rat hindlimbs. PTHrP was found to protect prostate carcinoma cells from apoptosis after phorbol myristate acetate (9). Effects on Fas-induced apoptosis are potentially relevant to lung cancer. Fas and Fas ligand have been reported to be positive prognostic factors for patients with lung cancer (20), and cis-platinum, topotecan, and gemcitabine induce apoptosis in lung cancer cell lines through death receptor-dependent mechanisms (10).

In summary, we have demonstrated that UV irradiation causes apoptosis of the BEN lung cancer cell line, as indicated by increased activities of caspase-3, caspase-8, and caspase-9, increased nuclear condensation, and decreased cell survival. Pretreatment with PTHrP-(1–34) or PTHrP-(140–173) attenuates the apoptotic effects of the radiation and increases cell survival in clonal survival assays. PTHrP-(140–173) also attenuates increases in activities of all three caspas after Fas ligation, but neither peptide has effects on caspase-3 or caspase-9 activities after staurosporine-induced apoptosis. These data indicate that the peptides protect BEN cells from apoptosis mediated by death receptor activation at or proximal to the action of caspase-8. UV-induced apoptosis in BEN cells apparently occurs through an extrinsic apoptosis pathway, possibly as a result of radiation-mediated aggregation of Fas receptor. The mechanism for the effects of the NH2-terminal and COOH-molecule PTHrP peptides on death receptor-mediated apoptosis in lung cancer cells requires further investigation. The clinical significance of these findings is under investigation.

DISCLOSURES

This work was supported by Department of Veterans Affairs Merit Review Grants (R. H. Hastings and L. J. Deftos), National Institutes of Health Grants ES-09227 (R. H. Hastings) and DK-60588 (L. J. Deftos), California Tobacco-Related Disease Research Program Grant 10RT-0161, and a grant from the Flight Attendants Medical Research Institute.

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