Protein Kinase Cα Is Activated by Caspase-dependent Proteolysis during Ultraviolet Radiation-induced Apoptosis of Human Keratinocytes*

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The elimination of ultraviolet (UV) radiation-damaged keratinocytes via apoptosis is an important mechanism for the protection of the skin from sunlight, an ubiquitous environmental carcinogen. Due to the pleiotropic nature of UV radiation, the molecular mechanisms of UV-induced apoptosis are poorly understood. Protein kinase C (PKC) is a family of enzymes critically involved in the regulation of differentiation in the epidermis, and is associated with the induction of apoptosis by ionizing radiation in other cell types. In normal human keratinocytes, the induction of apoptosis by UV exposure correlated with generation of the catalytic domain of PKCα in the soluble fraction. In contrast, phorbol ester 12-O-tetradecanoylphorbol-13-acetate caused translocation of PKCα from the soluble to the particulate fraction without inducing apoptosis. The effect of UV radiation on PKCα was isoform specific, as UV exposure did not stimulate the cleavage, or effect the subcellular distribution of any other PKC isoform. The soluble, catalytic domain of PKCα induced by UV exposure was associated with an increase in soluble PKCα activity. Proteases of the caspase family are activated during UV-induced apoptosis. In addition, inhibition of PKC activity specifically inhibited UV-induced apoptosis of keratinocytes, without affecting the G0/G1 cell cycle block induced by UV exposure. These results indicate that PKC activation is involved in the UV-induced death effector pathway of keratinocytes undergoing apoptosis, and defines a novel role for this enzyme in epidermal homeostasis.

Programmed cell death, or apoptosis, is a protective cellular response to genotoxic insults or environmental stress mediated by chemotherapeutic drugs, loss of cell-matrix adhesion, or ionizing radiation (1, 2). UV radiation from the sun is an inducer of keratinocyte apoptosis, and is the major etiological agent for the formation of multiple types of skin cancers, acting as both an initiating agent and a tumor promoter (3). While much is known regarding the ability of UV exposure to cause DNA mutations, little is known about the regulation of apoptosis in keratinocytes exposed to UV radiation. Multiple signaling pathways appear to be involved in the apoptosis of keratinocytes following UV exposure, including production of tumor necrosis factor α, and activation of CD95 (Fas/APO-1) (4–7). In addition, exposure of keratinocytes to UV radiation activates the caspase family of cysteine proteases, important mediators of the apoptotic program (4, 8).

PKCα is a family of serine/threonine protein kinases involved in the signal transduction of many growth factor receptors, and more recently implicated in apoptosis in a variety of cell types. At least 11 isoforms of PKC have been identified, and can be classified into classical (α, β, βII, γ), novel (δ, ε, η, θ, µ), and atypical (ζ, η′) families based upon their co-factor requirements (9, 10). The activities of atypical PKC isoforms (ζ, η′) are inhibited by UV exposure, and overexpression of an atypical PKC isoform can protect against UV-induced apoptosis (11, 12). Atypical PKC isoforms have also been implicated in the activation of AP-1 by UV light (13). Ceramide, an important second messenger generated in response to many apoptotic stimuli (14, 15), induced the redistribution of PKCα and ε from particulate to cytosol fraction in human leukemia cells. Both PKCα and PKCs and translocation and apoptosis was inhibited by TPA (16). In addition, multiple PKC isoforms (α, βI, δ, ε, θ, and ζ) can undergo limited proteolysis in cells undergoing apoptosis (17–20). The proteolysis of PKC in apoptotic cells generates a constitutively active catalytic domain, free from the normal inhibitor regulatory domain. The cleavage site has been identified as a caspase-3 (CPP32) recognition sequence (DMQD330/N for PKCα) in the hinge region (V3) of PKCα, and the proteolysis of PKCα can be blocked by peptide caspase-3 inhibitors, or the anti-apoptotic proteins Bcl-2 or Bcl-x (17, 19–22). Furthermore, expression of the catalytic domain of PKCα in HeLa, NIH3T3, and COS1 cells is sufficient to induce apoptosis, suggesting that PKCα may be an important effector of apoptosis (19, 21).

PKCα is also a central regulator of the keratinocyte maturation program (23–25). Keratinocytes express 5 PKC isoforms (α, δ, ε, η, and ζ) (24, 26), however, PKCη is only expressed in differentiating keratinocytes (27). Activation or increased expression of PKCη and PKCζ is linked to the differentiation program (24, 25, 27, 28). In addition, the classic and novel PKC isoforms are the molecular target for tumor promoting phorbol esters in skin chemical carcinogenesis models (29, 30). Due to the roles of PKC in apoptosis, epidermal differentiation, and carcinogenesis, we examined the effects of UV exposure on PKC isoforms in human keratinocytes. Our results demonstrate that PKCα is activated by UV radiation, and that inhibition of PKC significantly inhibits apoptosis induced by UV radiation.

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1 The abbreviations used are: PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate; PBS, phosphate-buffered saline; PARP, poly(ADP-ribose) polymerase; AEBSF, 4-(2-aminoethyl)-benzenesulfonyl fluoride.
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**EXPERIMENTAL PROCEDURES**

**Antibodies and Chemicals**—For detection of PKC isoforms, all antibodies were tested for specificity on a blot containing 50 ng of purified PKC isoforms (\(\alpha, \beta, \beta I, \gamma, \delta, \epsilon, \zeta\)) (Pan Vera Corp., Madison, WI) and PKC\(\eta\) (Upstate Biotechnology, Inc., Lake Placid, NY). The following antibodies were used to detect PKC\(\eta\) at 1:2000 and PKC\(\eta\) at 1:2000 (05–154 and 06–605, Upstate Biotechnology Inc., Lake Placid, NY); PKC\(\delta\) at 1:4000 (sc-937, Santa Cruz Biotechnology, Santa Cruz, CA); PKC\(\eta\) at 1:500 (P2279, Pan Vera Corp., Madison, WI); and PKC\(\zeta\) at 1:250 (sc-7272, Santa Cruz Biotechnology). The anti-caspase-3/CPP32 antibody was used at 1:200 (C31720, Transduction Labs, Lexington, KY), and the anti-PARP antibody was used at 1:10,000 (8192-1, CLONTECH, Palo Alto, CA).

The bisindoylmaleimide PKC inhibitors (I, IV, and V) were purchased from Alexis Biochemicals (San Diego, CA). The caspase inhibitor DEVD-FMK or z-DEVD-FMK was from CLONTECH Laboratories, Inc. or Enzyme Systems Products (Livermore, CA). The caspase inhibitor z-VAD-FMK was from Enzyme Systems Products. The caspase inhibitor YVAD-CMK was from Calbiochem (La Jolla, CA) and CLONTECH Laboratories, Inc. All inhibitors were dissolved in dimethyl sulfoxide.

**Cell Culture and UV Treatment**—Normal human epidermal keratinocytes were isolated from neonatal foreskins following routine circumcision (31, 32). The foreskins were stored for 1–2 days at 4 °C in Earl's balance salt solution containing 400 units/ml penicillin-streptomycin and 10 units/ml Nystatin. Foreskins were cut into several pieces, rinsed with PBS, lyses in PKC lysis buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 40 \(\mu\)g/ml leupeptin, 1 \(\mu\)M pepstatin, 1 mM AEBSF), and spun at 100,000 \(\times\) g for 1 h at 4 °C. The supernatant was removed as the soluble fraction, and the pellet extracted with PBS and scraped into PKC lysis buffer with 1% Triton X-100. The lysates were spun in a microcentrifuge for 10 min at 4 °C, and 30 \(\mu\)g of protein from the supernatant run on a SDS-polyacrylamide gel. The proteins were transferred to nitrocellulose and stained with Ponceau S to ensure equal protein loading. The membranes were blocked with 5% milk in 20 mM Tris-HCl, pH 7.6, 150 mM NaCl (TBS), stained with primary and horseradish peroxidase-conjugated secondary antibodies for 1 h each, and washed extensively in TBS, 0.05% Tween 20. Proteins were detected using the ECL detection kit (Amersham Life Sciences).

**Gel Electrophoresis DNA Analysis**—Low molecular weight DNA was analyzed as an alternative measure of apoptosis in some experiments. Floating and attached cells were lysed in 5 mM Tris-HCl, pH 7.5, 20 mM EDTA, 0.5% Triton X-100 and spun in a microcentrifuge at 4 °C for 30 min. The supernatant was extracted twice with phenol/chloroform/isoamylalcohol, once with chloroform, and the low molecular weight nucleic acids precipitated overnight at -20 °C with 0.1 volumes of 3 M sodium acetate and 2.5 volumes 100% ethanol. The pellet was suspended in 20 \(\mu\)l of 50 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 60 \(\mu\)g/ml RNase A, and incubated at 37 °C for 30 min. The DNA was then run on a 1.5% agarose gel and visualized with ethidium bromide.

**Flow Cytometry**—For cell cycle and apoptosis analysis, DNA content was measured by propidium iodide staining and flow cytometry (33). After treatments, keratinocytes were trypsinized and combined with floating cells, counted, and 10\(^6\) cells washed once in 2 ml of fluorescence-activated cell sorter buffer (PBS, 5% fetal bovine serum, 0.02% sodium azide). The cell pellet was suspended in 100 \(\mu\)l of fetal bovine serum on
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Fig. 2. UV dose response for the generation of PKCδ catalytic fragment. Keratinocytes from parallel dishes of Fig. 1 were irradiated with the indicated doses of UV radiation and cell lysates prepared after 18 h. 30 µg of protein was loaded per lane and blotted with the indicated PKC antibody. The arrows indicate the full-length PKC isoforms. Note the induction of the PKCδ catalytic domain cleavage product at about 42 kDa by 20–40 mJ/cm² UV radiation, as denoted by the asterisk. PKCγ was not detected and is not shown.

A

B

FIG. 3. Time course for the induction of apoptosis and cell cycle arrest in human keratinocytes by UV exposure. Keratinocytes were irradiated with 30 mJ/cm² UV radiation, stained with propidium iodide after the indicated times, and DNA quantified by flow cytometry. A, the DNA histograms show the accumulation of apoptotic cells (sub-G₀ DNA content) after 18–24 h. The percentage of cells with sub-G₀ DNA is indicated. B, the graph shows an increase in the percentage of cells with sub-G₀ DNA content, as well as an increase in G₀/G₁ cells 18–24 h after UV exposure. The data was quantified from the histograms in A as described previously.

RESULTS

Time and Dose-dependent Induction of Apoptosis and PKCδ Cleavage in Human Keratinocytes—Normal human epidermal keratinocytes were exposed to 0–40 mJ/cm² UV light, and after 18 h their DNA content measured to assay for changes in cell cycle and the induction of apoptosis. The histograms in Fig. 1A show that while no sub-G₀ DNA was detected in 0–10 mJ/cm² UV-irradiated cells, 20–40 mJ/cm² UV exposure induced the appearance of a significant number of cells with sub-G₀ DNA content, characteristic of apoptotic cells. The increase in cells with sub-G₀ DNA was quantitated (M1 region) and reached a maximum of 16.5% at 40 mJ/cm², as shown in Fig. 1B. Another major cellular response of normal cells to UV exposure is the arrest of cells in the G₁ phase of the cell cycle to allow for repair of DNA damage. Exposure of normal human keratinocytes to UV radiation induced a G₁ arrest, although the G₁ arrest was evident at low doses of UV exposure (10 mJ/cm²) which did not induce significant apoptosis (Fig. 1B). Apoptosis is also associated with endonuclease activation and the generation of low

ice, and 600 µl of ice-cold 100% ethanol added with gentle vortexing. The cells were incubated for 30 min on ice and washed once with fluorescence-activated cell sorter buffer. The cells were suspended in 0.5 ml of 10 µg/ml RNase in PBS and incubated at 37 °C for 15 min. After 5 min at room temperature, 0.5 ml of 100 µg/ml propidium iodide in PBS was added to each sample, mixed gently, and incubated at 4 °C for at least 1 h. Propidium iodide staining was quantitated by running the samples on a Coulter Epics XL-MCL flow cytometer. Cells with DNA content less than the G₀/G₁ amount of untreated cells were considered apoptotic. For cell cycle analysis, DNA histograms were analyzed using MultiCycle for Windows (Phoenix Flow Systems, San Diego, CA).

PKCδ Immunoprecipitation Kinase Assays—Cells were lysed in immunoprecipitation lysis buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM EGTA, 100 mM ATP, and 1 µCi of [γ-³²P]ATP. Reaction was incubated for 10 min at 30 °C, placed on ice, and spun in a microcentrifuge briefly to pellet the agarose. 25 µl of supernatant was spotted onto P81 phosphocellulose disks, washed, and radioactivity counted.

Enzyme activity was assayed as described previously (34, 35) with minor modifications. Assays were performed in 30 µl of assay buffer containing 33 µM PKCα peptide substrate (Ser²²) (Life Technologies, Inc., Gaithersburg, MD), 330 µM ATP, and 10 µCi of [γ-³²P]ATP. Reactions were incubated for 10 min at 30 °C, placed on ice, and spun in a microcentrifuge briefly to pellet the agarose. 25 µl of supernatant was spotted onto P81 phosphocellulose disks, washed, and radioactivity counted.
molecular weight DNA, which can be visualized as a DNA ladder. UV radiation induced the appearance of the low molecular weight DNA ladder at a dose (30 mJ/cm²) which also induced sub-G₀ DNA (Fig. 1C).

Several PKC isoforms have been implicated in the apoptotic response of cells to variety of stimuli which induce programmed cell death (9, 10, 17–20). We examined the levels of all PKC isoforms expressed in keratinocytes exposed to 0–40 mJ/cm² UV light to determine if UV light influenced specific PKC isoforms in keratinocytes undergoing apoptosis. UV light induced the generation of a specific ~42 kDa cleavage product of PKCδ at doses of 20–40 mJ/cm², the doses which induced apoptosis (Fig. 2). The cleavage product corresponds in size to the catalytic domain of PKCδ. The catalytic domain of PKCδ induced by UV radiation was often detected as a doublet, with the upper band being darker (Figs. 2, 4, 5, and 7). The redistribution of PKC from the soluble fraction to the particulate fraction was sometimes observed for PKCα, PKCe, or PKCζ after UV exposure and following UV exposure (data not shown).

A time course of the effects of UV radiation on DNA content in keratinocytes is shown in Fig. 3, A and B. A large increase in the number of apoptotic cells with sub-G₀ DNA content was observed 18–24 h after UV exposure, concomitant with an increase in G₀/G₁ cells and a decrease in S and G₂/M cells. The kinetics of proteolytic cleavage of PKCδ paralleled the increase in apoptotic cells at 18–24 h (Fig. 4). Although not detected in this experiment, the PKCδ catalytic domain was sometimes detected 8 h after UV exposure and thus was variable between experiments. No other changes in PKCα, PKCe, or PKCζ were reproducibly observed following UV exposure, except for a slight upward shift in migration of PKCe after 18–24 h.

**Activation of PKCδ in the Soluble Fraction by UV Radiation**—The redistribution of PKCδ from the soluble fraction to the particulate fraction is a useful indicator of PKC activation by phorbol esters or endogenous lipophilic activators (9). We measured the subcellular distribution of PKC isoforms in keratinocytes at 0, 0.5, 6, and 20 h after 30 mJ/cm² UV exposure (Fig. 5). There was no translocation detected of any PKC isoform (α, δ, ε, or ζ) at any time point. In contrast, a 30-min treatment with 500 nM TPA caused the redistribution of PKCα and PKCδ from the soluble to the particulate fraction. The catalytic domain cleavage product of PKCδ was present only in the soluble fraction 20 h after UV irradiation.

**Inhibition of UV-induced Apoptosis and PKCδ Cleavage by Caspase Inhibitors**—The proteolytic cleavage and subsequent activation of PKCδ may be mediated by members of the caspase family of cysteine proteases activated during UV-induced apo-
ptosis, particularly caspase-3 which is the only known caspase capable of cleaving PKCδ (21). To test this hypothesis, we pretreated keratinocytes with the peptide caspase inhibitors DEVD, z-VAD, and YVAD and exposed them to UV radiation. DEVD and z-VAD are effective inhibitors of caspase-3 activity, while YVAD is a very weak inhibitor of caspase-3 (36, 37). Fig. 7A shows that DEVD and z-VAD blocked UV-induced apoptosis by 80 and 90%, respectively, while YVAD did not protect against UV-induced apoptosis. UV-induced apoptosis also induced the proteolysis of caspase-3, which is activated by proteolytic processing (36), and this proteolytic cleavage was partially inhibited by DEVD, but not by YVAD (Fig. 7B). In addition, z-DEVD, but not YVAD, blocked the UV-stimulated cleavage of the caspase-3 substrates PKCδ and poly(ADP-ribose) polymerase (PARP) (Fig. 7B). Thus the inhibition of caspase-3 proteolytic processing by caspase inhibitors correlated with a block in caspase-3 activation.

**Inhibition of PKC Blocks UV-induced Apoptosis, but Not G1 Arrest**—To determine if PKC activation is functionally important for UV-induced apoptosis, we tested the ability of several bisindolylmaleimide PKC inhibitors to block apoptosis of UV-irradiated keratinocytes. Fig. 8A shows that bisindolylmaleimide I (GF 109203X) inhibited 75% of the apoptosis induced by UV exposure. GF 109203X and TPA alone did not induce apoptosis. GF 109203X also blocked the DNA fragmentation and morphological cell death induced by UV exposure (Fig. 8, B and C). We also determined if PKC inhibition could block the UV-induced G1 arrest. Shown in Fig. 8D, GF 109203X alone caused a slight increase in S phase cells, and was unable to block the G1 arrest induced by UV exposure. In contrast, GF 109203X completely inhibited the G1 arrest induced by TPA.

Some of the effects of UV exposure on cells are due to its interaction with cellular membranes resulting in growth factor receptor clustering and activation, lipid peroxidation, and the generation of reactive oxygen species (14, 38). To determine if GF 109203X was blocking apoptosis by some general or non-specific membrane effect, we tested the ability of two structurally related bisindolylmaleimides to inhibit UV-induced apoptosis. Bisindolylmaleimide IV is active as a PKC inhibitor while bisindolylmaleimide V does not inhibit PKC, and differs from bisindolylmaleimide IV by only a single methyl group. Bisindolylmaleimide IV inhibited apoptosis induced by UV exposure,

**Fig. 6. Activation of PKCδ in the soluble fraction after UV irradiation.** Keratinocytes were irradiated with 30 mJ/cm² UV radiation, and after 22 h fractionated into total, soluble, and particulate fractions. PKCδ was immunoprecipitated and enzyme activity assayed. Note the induction of PKCδ activity by UV exposure in the total and soluble fractions, but not particulate fraction.

**Fig. 7. Inhibition of UV-induced apoptosis and caspase-3 activation by caspase inhibitors.** A, keratinocytes were pretreated with 10 μM of the indicated peptide for 15–30 min and irradiated with 30–40 mJ/cm² UV radiation. After ~18 h, cells were stained with propidium iodide and apoptotic cells (sub-G0 DNA content) quantified by flow cytometry. Data from two to four experiments was normalized so that the percentage of UV-induced apoptosis was 100%. Error bars indicate standard deviation. Note the inhibition of apoptosis by DEVD and z-VAD, but not by YVAD. B, lysates were prepared from parallel dishes treated as described in panel A and protein immunoblotted for caspase-3, PKCδ, and PARP. The full-length proteins are marked by the arrows, and the cleavage products of PKCδ and PARP are marked by an asterisk and a circle, respectively. Note the decrease in full-length 32-kDa caspase-3 by UV exposure and the protection from cleavage by DEVD, but not YVAD. The UV-induced cleavage of PKCδ and PARP was also protected by DEVD, but not YVAD.
while the inactive bisindolylmaleimide V failed to protect against apoptosis (Fig. 8E). This structure-activity relationship supports a specific role for PKC inhibition in blocking UV-induced apoptosis.

**DISCUSSION**

At the cellular level, the epidermis responds to the genotoxic and carcinogenic effects of UV radiation from sunlight by two major mechanisms: 1) arrest cells in the G1 phase of the cell cycle to allow time for repair of DNA damage, and 2) apoptosis of damaged cells to remove them from the tissue. We examined the involvement of the PKC family in both the cell cycle arrest and apoptotic response of normal human keratinocytes to UV exposure. UV radiation induced the proteolysis of PKC\(_d\), liberating the catalytic domain into the soluble fraction (Figs. 1–4). The appearance of PKC\(_d\) catalytic domain parallels the induction of apoptosis, but did not occur at low doses of UV radiation (10 mJ/cm\(^2\)) which caused only G1 arrest (Figs. 1–4). Thus, G1 arrest of UV-irradiated keratinocytes is not dependent upon PKC\(_d\) proteolytic activation. In addition, the UV-induced G1 arrest of keratinocytes was not blocked by the PKC inhibitor GF 109203X, while TPA-induced G1 arrest was blocked by GF 109203X (Fig. 8A), further supporting the PKC-independent nature of UV-induced growth arrest. The G1 arrest of UV-irradiated keratinocytes is p53 dependent and does not occur in keratinocytes with mutant p53 (39, 40). p53 is also involved in the induction of apoptosis in cells exposed to UV radiation (3, 41). Fewer apoptotic or sunburned cells were formed in the epidermis of p53 \(-/-\) mice compared with p53 \(-/+\) or \(+/+\) mice, however, apoptotic cells were not completely absent in the p53 \(-/-\) epidermis, indicating a p53-independent pathway for the induction of apoptosis in irradiated keratinocytes (3, 42).

The catalytic domain of PKC\(_d\) induced by UV radiation was in the soluble fraction, corresponding with the increase in soluble PKC\(_d\) activity (Figs. 5 and 6). In control, proliferative cultures, PKC\(_d\) was distributed ; 55% in the soluble fraction and ; 45% in the particulate fraction (Fig. 5). Full-length, soluble PKC is considered enzymatically inactive since lipophilic activators induce the translocation of PKC into the particulate

**Fig. 8. Inhibition of UV-induced apoptosis by PKC inhibitors.** A, keratinocytes were irradiated with 30–40 mJ/cm\(^2\) UV, or treated with 500 nM TPA with or without 5 \(\mu\)M GF 109203X (GF) as indicated. For UV radiation treatments, GF 109203X was added after irradiation. For TPA treatment, keratinocytes were pretreated for 10 min with GF 109203X. After ~18 h, cells were stained with propidium iodide and apoptotic cells (sub-G\(_0\) DNA content) quantified by flow cytometry. Data from two to three experiments was normalized so that the percentage of UV-induced apoptosis was 100%. Error bars indicate standard deviation. B, low molecular weight DNA was isolated from untreated cells, cells irradiated with 30 mJ/cm\(^2\) UV, or irradiated cells cultured with 5 \(\mu\)M GF 109203X (GF). Note the inhibition of UV-stimulated DNA fragmentation by GF 109203X. C, phase-contrast picture of untreated keratinocytes, keratinocytes irradiated with 30 mJ/cm\(^2\) UV radiation with or without GF 109203X as described in panel A. Note the protection from UV-induced cell death by GF 109203X (UV + GF). D, cell cycle analysis was performed on cells treated as described in panel A. The percentage of cycling cells in either G1, S, and G2/M is shown. Note the increase in G1/S, G1/G0 cells by both UV and TPA, but GF 109203X (GF) blocks only the TPA-induced cell cycle arrest. E, keratinocytes were irradiated with 30 mJ/cm\(^2\) UV and treated with 5 \(\mu\)M bisindolylmaleimide IV (Bis. IV) or bisindolylmaleimide V (Bis. V), as indicated. After ~23 h, cells were stained with propidium iodide and apoptotic cells (sub-G\(_0\) DNA content) quantified by flow cytometry. Data from two experiments was normalized so that the percentage of UV-induced apoptosis was 100%. Error bars indicate standard deviation. Note that Bis. IV inhibited UV-induced apoptosis, but the inactive Bis. V did not.
fraction, as we observed with TPA (Fig. 5), and the translocation to the particulate fraction is often used as an indicator of activation (9). The increase in soluble PKCδ activity by UV radiation is unique, and may afford a unique substrate accessibility to the catalytic domain of PKCδ. TPA induced an accumulation of cells in G2/M, most likely due to the induction of differentiation (43), but did not induce apoptosis (Fig. 8). UV exposure induces expression of some differentiation markers and suppresses expression of others, indicating a complex effect of UV radiation on epidermal differentiation and homeostasis (44–46). In addition to the different subcellular localization of activated PKC, UV exposure, and TPA had different PKC isoform activation specificity and kinetics of activation. TPA caused the rapid (30 min) translocation of PKCα and PKCδ, but UV exposure did not induce translocation of any isoform. These differences in PKC isoform activation and subcellular distribution may explain some differences between the cellular effects of TPA and UV radiation (19). A shift to a slower migrating band was observed for PKCe, however, the nature of the slower migrating PKCe is unknown. Although no changes in the levels or subcellular distribution were observed for any PKC isoform except PKδ, we cannot rule out the possibility that other PKC isoforms are also influenced by UV radiation in keratinocytes.

Inhibition of caspases with DEVD or z-VAD blocked UV-induced apoptosis, while YVAD did not block apoptosis (Fig. 7A). DEVD inhibits caspase-3 with a Ki of 0.52 nm, while YVAD has a Ki of 500 μM for caspase-3 inhibition (37). The efficiencies of these peptide caspase inhibitors for a particular caspase are not absolute, however, their ability to inhibit caspase-3 in vitro correlates with the ability to inhibit UV-induced apoptosis (Fig. 7) (36, 37). In addition, YVAD did not prevent UV-induced cleavage of caspase-3, or its substrates PKCδ and PARP, while DEVD did partially block the cleavage of caspase-3, PKCδ, and PARP. YVAD, a potent inhibitor of caspase-1/ICE, was also much less effective than DEVD or z-VAD at inhibiting anti-Fas-induced apoptosis of Jurkat cells (Ref. 47, and data not shown). These results with YVAD suggest that caspase-1 activation is not required for the induction of apoptosis by UV radiation in keratinocytes. The inhibition of PKCδ cleavage by caspase inhibitors suggests that PKCδ cleavage is downstream of caspase activation. However, the PKC inhibitor GF 109203X also inhibited the cleavage of PKCδ by UV exposure, raising the possibility that PKC activation may also be required for caspase activation (data not shown).

We also observed inhibition of UV-induced apoptosis by PKC inhibitors of the bisindolylmaleimide class (Fig. 8). The extent of inhibition was greater than 50%, but never complete, suggesting the presence of an alternative pathway independent of PKC activation. The stage of the cycle a cell is in can influence its sensitivity to radiation, however, the GF 109203X did not alter the cell cycle distribution of the keratinocytes (Fig. 5D). Inhibition of PKC completely reversed the G1 arrest induced by TPA, indicating that the GF 109203X was active at the dose used (5 μM). The bisindolylmaleimide PKC inhibitors are competitive with ATP (48, 49), and thus are active against the free catalytic domain of PKCδ. In addition, the structure-activity relationship of bisindolylmaleimide IV and V for PKC inhibition correlated with apoptosis inhibition. We also pretreated keratinocytes with either Brystatin I or TPA to down-regulate PKC, and examined the ability of cells to undergo UV-induced apoptosis, but our results were variable (data not shown). TPA enhanced the induction of apoptosis by interferon-γ and anti-Fas antibody in transformed human keratinocytes (50). Pretreatment with TPA did reduce the number of apoptotic cells induced by UV exposure in an in vitro skin equivalent model system, however, PKC levels were not examined in this study and TPA may cause other cellular or biochemical changes in the keratinocytes which alter their sensitivity to apoptosis (51).

The induction of apoptosis by UV exposure is believed to be a protective mechanism for the epidermis. Targeted expression of the anti-apoptotic protein Bcl-xL to the epidermis of mice conferred resistance to UV-induced apoptosis and increased susceptibility to tumor induction by chemical carcinogens (52, 53). Thus, the elimination of UV-induced DNA damaged keratinocytes via apoptosis reduces the risk of developing skin cancer.

PKC has been under intense study since it was recognized that it is the receptor for phorbol ester tumor promoters used in chemical carcinogenesis studies (29, 30). PKC also plays an essential role in the regulation of the ordered, stepwise keratinoctye differentiation program within the epidermis (23–25), and disruption of the differentiation/proliferation homeostasis by phorbol esters may contribute to its carcinogenic effects. Our results demonstrate that PKC is also an important molecular target for UV light, an inducer of apoptosis and the major human skin carcinogen. Thus, PKC is important in the signaling of both chemical and photo-carcinogens. A more thorough understanding of the signaling pathways involved in epidermal keratinocyte cell death by UV exposure may provide insight into the development of protective interventions for photocarcinogenesis.

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