Fas-mediated Apoptosis in Human Prostatic Carcinoma Cell Lines Occurs via Activation of Caspase-8 and Caspase-7

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ABSTRACT

We previously demonstrated that treatment with cycloheximide (CHX) converted the phenotype of Fas-resistant human prostatic carcinoma cell lines to Fas-sensitive and that resistance to Fas-mediated apoptosis was due to a dominant-negative protein(s). In this study, we investigated the sequential activation of caspase family members, to gain insight into the likely site of action of the suppressor protein(s). We did not find Tyr-Val-Ala-Aspase activity in any of the cell lines examined. Time-dependent Asp-Glu-Val-Ala-Aspase activity was detected during Fas-mediated apoptosis in Fas-sensitive cell lines PC3 and ALVA31. Asp-Glu-Val-Ala-Aspase activity in Fas-resistant cell lines DU145 and JCA1, was detected only under combined treatment with CHX and anti-Fas agonistic mAb. In experiments with caspase inhibitors we show that Fas-mediated apoptosis in PC3 is mainly executed by the caspase-3 subfamily, but another member(s) of the caspase family may be involved in Fas-mediated apoptosis in ALVA31, DU145, and JCA1. Western blot analysis revealed that Fas-ligation activated caspase-7, but not caspase-3. The activated form of caspase-8 was detected in DU145 only after 4 h of simultaneous treatment with CHX and anti-Fas mAb, whereas in PC3 caspase-8 was found to be activated after 1 h of Fas-ligation. We have also found that treatment with staurosporin did not activate caspase-8, whereas staurosporin induced apoptosis in both Fas-resistant and Fas-sensitive cell lines. These results suggest that an inhibitory protein(s), which suppresses apoptosis in Fas-resistant cell lines, presumably acts at the apex of apoptotic cascade by preventing the activation of caspase-8.

INTRODUCTION

Execution of apoptosis in eukaryotic cells is an active biochemical process that depends upon activation of proenzymes of the aspartate-specific cysteine protease family (caspases; reviewed in Refs. 1–3). Comparative analysis of the caspases reveals the existence of three subfamilies: an ICE-like (3) subfamily (comprising caspases-1, -4, and -5), a CPP32 subfamily (comprising caspases-3, -6, -7, -8, -9, and -10), and an ICH1-Needd2 (caspase-2) subfamily. Although members of the ICE-like and CPP32-like subfamilies have different substrate specificity, all caspases are cleaved at specific Asp residues and, based on that observation, a model of hierarchical activation of caspases has been proposed (4). In this model, caspase-8 has been termed an initiator protease, which activates executioner proteases such as caspase-3 or caspase-7. Once procaspase-3 is activated by upstream caspases, it could activate procaspase-6, which in turn may feed back on procaspase-8, resulting in a protease amplification cycle. It has been also suggested that activation of the upstream caspase-8 and -10 may be dependent on autocatalysis, which is triggered by interaction with FADD or FADD-like death-effector proteins that receive the apoptotic signal from Fas- or TNF-α-receptors (5). The mode of activation of caspase-8 remained unclear because caspase-8 represents the most proximal caspase in the Fas- and TNF-α-mediated apoptosis. However, it has recently been shown that clustering of procaspase-8 to Fas-FADD complex causes activation of caspase-8 due to an intrinsic proteolytic activity of the zymogen (6).

We have previously shown that of six human prostatic carcinoma cell lines investigated (ALVA31, DU145, JCA1, LNCaP, ND1, and PC3), agonistic anti-Fas antibody induced apoptosis in only two, PC3 and ALVA31. However, treatment with CHX converted the phenotype of Fas-resistant cell lines DU145 and JCA1 to Fas sensitive (7). Subsequently, we generated somatic hybrids between Fas-resistant and Fas-sensitive cell lines and found that hybrid cells were resistant to apoptosis (8). Thus, resistance to Fas-mediated apoptosis is thought to be an active process mediated by a labile protein(s), and dominates over sensitivity. However, the individual caspases responsible for Fas-mediated apoptosis in prostatic carcinoma cell lines have not been identified. In addition, the hierarchy of caspase activation is unknown.

In this study, we investigated the possible involvement of caspase subfamilies in Fas- and TNF-α-mediated apoptosis in human prostatic carcinoma cell lines. To perform these experiments, we used ICE-like- (YVAD) and CPP32-like (DEVD)-specific substrates, five different cell-permeable inhibitors of caspases and mAbs specific to both proenzyme and the large subunits of caspase-8, -3, and -7. Our data indicate that the CPP32-like subfamily is involved in Fas-mediated apoptosis in PC3 and ALVA31 and in TNF-α-mediated apoptosis in PC3. However, none of the inhibitors used completely prevented TNF-α-mediated apoptosis in ALVA31. These results, therefore, indicate that in addition to caspases another protease(s) may participate in the execution of TNF-α-mediated apoptosis in ALVA31. We have also demonstrated that caspase-8 is activated first during Fas-mediated apoptosis, which in turn induced activity of caspase-7. Importantly, we observed the same hierarchy of caspase activation in Fas-sensitive and Fas-resistant cell lines under treatment with CHX and Fas-ligation. Taken together, these results indicate that an inhibitor protein(s) in Fas-resistant cell lines act upstream possibly inhibiting the activation of caspase-8.

MATERIALS AND METHODS

Cell Culture. The various human prostatic carcinoma cell lines were described previously (7). Cells were cultured in RPMI 1640 supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 1 mM sodium pyruvate, 10% heat-inactivated FCS (HyClone Laboratories), 0.1 mM 2-mercaptoethanol, and 2 mM L-glutamine. Cells were subcultured at 1:10 dilution by trypsinization for 2–4 min at room temperature.

Quantitative DNA Fragmentation Assay. The method was described previously (7). Briefly, to quantitate DNA lost during apoptosis, cells were prelabeled overnight with [3H]thymidine and then cultured for various times in 96-well flat-bottomed plates (5–10,000 cells/well) in the presence of different concentrations of TNF-α and anti-Fas mAb (IPD-4). In separate experiments, cells were treated with kinase and phosphatase inhibitors: okadaic acid, sodium orthovanadate, staurosporin, and genistein (Sigma Chemical Co.). The radioactivity was measured by liquid scintillation counting in triplicate or sextuplets.
plicate samples. The percentage of DNA fragmentation was calculated as follows:

\[
\text{cpm in untreated group} - \text{cpm in treated group} \times 100
\]

Inhibition of Apoptosis. To investigate the role of different inhibitors in apoptosis, the DNA fragmentation assay was performed in the presence of five different cell-permeable inhibitors of caspases: Z-VAD-FMK, Ac-YVAD-CMK, Z-DEVD-FMK, and Z-VDVAD-FMK (all from Calbiochem), and Ac-IETD-CHO (BIOMOL). Inhibitors and apoptosis inducers (anti-Fas mAb or TNF-α) were added at the time of plating, and apoptosis was measured by DNA fragmentation assay.

Assay of Caspase Activity. To measure caspase activity, cytosolic extracts were prepared as described (9) by repeatedly freezing and thawing of cells in KPM buffer (50 mM PIPES-NaOH (pH 7.0), 50 mM KCl, 10 mM EGTA, 1.92 mM MgCl2, 1 mM DTT, 10 μM/ml cysteine B, and 2 μg/ml pepstatin, leupeptin, and antipain). Protein lysate (25–50 μg) was incubated at room temperature for 30, 60, and 90 min in assay buffer (50 mM PIPES-KOH (pH 7.0), 0.1 mM EDTA, and 10% glycerol) with 20 μM fluorescent substrates: Ac-DEVD-AMC as a CPP32-like caspase substrate, and Ac-YVAD-AMC as an ICE-like caspase substrate. Fluorescence at 360/460 nm was measured with a FL500 fluorometer (Bio-Tek Instruments, Inc.). Measurements were calibrated according to a standard curve of AMC (Sigma Chemical Co.), and data were expressed in nm of released AMC/μg of lysate proteins.

Western Blotting. Western blot detection of proteins was performed as described previously (7). Briefly, protein concentrations of cytosolic proteins were measured with the Bio-Rad DC protein microassay, 15–25 μg of proteins were separated on 4–20% gradient SDS-PAGE, and blotted to nitrocellulose membrane (Novex). Membranes were blocked with 5% nonfat dry milk in PBS containing 0.1% Tween 20 and then incubated with mouse mAbs to caspase-3, -7, and -8 (FLICE). mAbs against caspases were kindly provided by Dr. Yuri Lazebnik (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). The blots were counterstained with goat antimouse IgG conjugated with horseradish peroxidase. The immunoreactive bands were visualized by incubation of the membrane with enhanced chemiluminescence reagent (Pierce Chemical Co.). In separate experiments, total cell extracts were prepared using Laemmli’s sample buffer and blots were incubated with mAb to PARP (PharMingen) and Rb (Ab-5; Oncogene Science).

RESULTS

DEVDase, but not YVADase, Activity Is Induced in Prostatic Carcinoma Cells during Fas- and TNF-α-mediated Apoptosis. Cytosolic extracts were prepared from prostate cells that were treated with 1 μg/ml anti-Fas mAb or 20 ng/ml TNF-α for various times. ICE- and CPP32-like activity in the lysates (25–50 μg of protein) was then determined using the fluorescent substrates Ac-YVAD-AMC or Ac-DEVD-AMC, respectively. As shown in Fig. 1, we did not find ICE-like activity in any of the cell lines examined under treatment with anti-Fas mAb and TNF-α. However, we detected time-dependent CPP32-like activity during Fas-mediated apoptosis in Fas-sensitive cell lines (PC3 and ALVA31), and in Fas-resistant cell lines (JCA1 and DU145) under combined treatment with CHX and anti-Fas mAb (Fig. 1). We also noted that DEVDase activity was induced at low levels under TNF-α treatment in PC3, but especially in ALVA31, when compared with Fas ligation. Importantly, in Fas-resistant cell lines DU145 and JCA1, treatment with CHX alone or with anti-Fas mAb alone did not induce caspase activity, and only simultaneous treatment with CHX and anti-Fas mAb induced DEVDase activity.

Inhibition of Apoptosis by Caspase Inhibitors. Because anti-Fas mAb and TNF-α induced CPP32-like proteolytic activity, we investigated whether caspase activation is central in Fas- and TNF-α-mediated apoptosis. The ability of anti-Fas mAb and TNF-α to induce apoptosis was investigated in the presence of five caspase inhibitors. As shown in Fig. 2, incubation of PC3 cells with Z-VAD-FMK, a peptide caspase inhibitor with broad specificity, completely inhibited Fas-mediated apoptosis. The CPP32-like inhibitor, Z-DEVD-FMK, also completely inhibited apoptosis, although at a higher concentration than required for Z-VAD-FMK. The ICE-like inhibitor, Ac-YVAD-CMK, partially inhibited apoptosis, but was much less effective than the other two inhibitors. It has been previously reported that both ICE-like and CPP32 can cleave the YVAD and DEVD substrates, although ICE-like was more active than CPP32 in cleaving the YVAD substrate, but CPP32 was more active than the ICE-like caspase in cleaving the DEVD substrate (10). These data, therefore, can explain the low inhibitory activity of Ac-YVAD-CMK in PC3 despite the fact that ICE-like activity was not detected after anti-Fas treatment (Fig. 1). Both Z-VAD-FMK and Z-DEVD-FMK blocked TNF-α-mediated apoptosis in a similar concentration-dependent manner in PC3, and Ac-YVAD-CMK showed low inhibitory activity (Fig. 2). We have also used Z-VDVAD-FMK as a caspase-2 inhibitor (11) and Ac-IETD-CHO as a caspase-6 and caspase-8 inhibitor (11–13).

A different pattern of inhibition was found in ALVA31. Only Z-VAD-FMK was able to completely inhibit Fas-mediated apoptosis, whereas the maximal level of inhibition was 60% for Z-DEVD-FMK; Ac-YVAD-CMK, Z-VDVAD-FMK, and Ac-IETD-CHO showed minimal inhibition of apoptosis. Inhibition of TNF-α-mediated apoptosis in ALVA31 was found at low levels for all five caspase inhibitors (Fig. 2) and was consistent with low levels of caspase activation after TNF-α treatment (Fig. 1). The low level of inhibition of TNF-α-mediated apoptosis in ALVA31 by Z-VAD-FMK could not be explained by low level cell permeability of this inhibitor because Z-VAD-FMK was capable of completely inhibiting Fas-mediated apoptosis in ALVA31. Thus, these data suggest that in addition to the caspase family, a noncaspase protease(s) seems to be involved in TNF-α-mediated apoptosis in ALVA31. We have also found that Fas-
Western Blot Analysis Reveals Activation of Caspase-7 and Proteolysis of Rb and PARP. To investigate the role of individual caspases in Fas-mediated apoptosis, we used mAbs that reacted with caspase-1-, caspase-3-specific inhibitors and completely inhibited the pan-caspase inhibitor Z-VAD-FMK (Table 1).

In summary, Fas- and TNF-α-mediated apoptosis in PC3 seems to be executed by the caspase-3 subfamily. In addition to the caspase-3 subfamily, another member(s) of the caspase family could be involved in Fas-mediated apoptosis in ALVA31 and in Fas- and TNF-α-mediated apoptosis in DU145 and JCA1.

Western Blot Analysis Reveals Activation of Caspase-7 and Proteolysis of Rb and PARP. To investigate the role of individual caspases in Fas-mediated apoptosis, we used mAbs that reacted with both proenzyme and the large subunit of activated caspase-3,-7, and -8. Western blot analysis was performed on lysates of Fas-sensitive cell lines, PC3 and ALVA31, treated with 1 µg/ml anti-Fas mAb for 24 h in the presence of three caspase inhibitors. As shown in Fig. 3, Fas-ligation activated caspase-7; this was indicated by the appearance of a Mr 20,000 band, representing the active subunit. Caspase-3 remained in its inactive form and no changes were noticed in the intensity of the caspase-3 band during treatment with anti-Fas mAb. Caspase-7 cleavage was prevented by the addition of all three caspase inhibitors used in PC3 cells, but only Z-VAD-FMK prevented caspase-7 activation in ALVA31. These data are completely consistent with the apoptosis inhibition experiments that showed that these inhibitors were able to prevent apoptosis in PC3, but only Z-VAD-FMK was capable of inhibiting apoptosis in ALVA31 (Fig. 2). We also found activation of caspase-7, but not caspase-3, in Fas-resistant cell lines DU145 and JCA1 under combined treatment with CHX and anti-Fas mAb (data not shown). Fas-ligation also resulted in proteolysis of Rb and PARP. Proteolysis of Rb was prevented by all three caspase inhibitors in PC3, but in ALVA31 Rb proteolysis was not prevented by Ac-YVAD-CMK. PARP proteolysis was completely inhibited in PC3 by Z-DEVD-FMK and Z-VAD-FMK, but was partially inhibited by Ac-YVAD-CMK. In ALVA31, complete inhibition of PARP proteolysis was achieved only by using Z-VAD-FMK.

Thus, caspase-7, but not caspase-3, seems to participate in Fas-mediated apoptosis in Fas-sensitive cell lines PC3 and ALVA31. Moreover, proteolysis of Rb and PARP during Fas-mediated apoptosis was observed in both PC3 and ALVA31. However, the biochemical pathway that leads to activation of caspase-7 is different in these two cell lines because the effect of caspase inhibitors resulted in different patterns of proteolysis.

**Table 1** Inhibition of apoptosis by different caspase inhibitors in human prostatic carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Apoptosis induced by</th>
<th>Z-DEVD-FMK</th>
<th>Ac-YVAD-CMK</th>
<th>Z-VAD-FMK</th>
</tr>
</thead>
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<tr>
<td>PC3</td>
<td>Anti-Fas</td>
<td>100</td>
<td>42 ± 7</td>
<td>100</td>
</tr>
<tr>
<td>TNF-α</td>
<td>100</td>
<td>24 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALVA31</td>
<td>Anti-Fas</td>
<td>56 ± 6</td>
<td>22 ± 4</td>
<td>100</td>
</tr>
<tr>
<td>TNF-α</td>
<td>40 ± 8</td>
<td>20 ± 6</td>
<td>55 ± 8</td>
<td></td>
</tr>
<tr>
<td>DU145</td>
<td>CHX + A-Fas</td>
<td>57 ± 9</td>
<td>66 ± 5</td>
<td>100</td>
</tr>
<tr>
<td>CHX + TNF-α</td>
<td>50 ± 8</td>
<td>29 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JCA1</td>
<td>CHX + A-Fas</td>
<td>35 ± 7</td>
<td>41 ± 4</td>
<td>100</td>
</tr>
<tr>
<td>CHX + TNF-α</td>
<td>23 ± 6</td>
<td>14 ± 4</td>
<td></td>
<td></td>
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</tbody>
</table>

*Data are mean ± SD of measurements of apoptosis inhibition (indicated in percent of apoptosis inhibition) estimated by DNA fragmentation assay.*
Caspase-8 was found after 1 h of treatment with anti-Fas mAb. A low level of caspase-8 and caspase-7 were determined. As shown in Fig. 45, the level of activated caspase-7 was noted after 4 h of treatment in DU145 and PC3. The active form of caspase-8 was identified in DU145 only after 4 h of treatment with CHX and anti-Fas mAb, whereas in PC3 activated caspase-8 was not detected in DU145 and sharply decreased in PC3 (Fig. 4A). The opposite pattern was found for caspase-7. Low levels of the active form of caspase-7 were noted after 4 h of treatment, and the levels of activated caspase-7 increased after 8, 16, and 24 h of treatment (Fig. 4A). Because it was important to determine the initial time point of caspase activation, DU145 was treated with CHX alone, anti-Fas mAb alone and simultaneously with CHX and anti-Fas mAb. As shown in Fig. 4, caspase-8 and caspase-7 were activated in DU145 only under simultaneous treatment with CHX and anti-Fas mAb. The activation of these caspases was found to be time-dependent. The active form of caspase-8 was found after 4 h and 8 h of treatment both in DU145 and PC3, respectively. After 16 h of treatment, activated caspase-8 was not detected in DU145 and sharply decreased in PC3 (Fig. 4A). The opposite pattern was found for activation of caspase-7. Low levels of the active form of caspase-7 were noted after 4 h of treatment, and the levels of activated caspase-7 increased after 8, 16, and 24 h of treatment (Fig. 4A). Because it was important to determine the initial time point of caspase activation, DU145 was treated with CHX and anti-Fas mAb, and PC3 was treated with anti-Fas mAb for 15 min, 30 min, 1 h, 2 h, and 4 h, and activation of caspase-8 and caspase-7 were determined. As shown in Fig. 4B, the active form of caspase-8 was identified in DU145 only after 4 h of treatment with CHX and anti-Fas mAb, whereas in PC3 activated caspase-8 was found after 1 h of treatment with anti-Fas mAb. A low level of activated caspase-7 was noted after 4 h of treatment in DU145, whereas in PC3 the active form of caspase-7 was found after 2 h of treatment (data not shown).

Thus, Fas-ligation in the cell lines examined results in the activation of caspase-8 that, presumably in turn, activates caspase-7. In DU145, only simultaneous treatment with CHX and anti-Fas mAb activated caspase-8. These data suggest that the effect of a regulatory protein(s), which is responsible for resistance to Fas-mediated apoptosis, lies at the apex of the apoptotic cascade.

Staurosporin Activates Caspase-7, but not Caspase-8 in Both Fas-sensitive and Fas-resistant Cell Lines. In a previous study, it has been suggested that caspase-8 mediates Fas- and TNF-α-apoptotic pathways, whereas caspase-10 mediates apoptotic pathways induced by DNA-damaging agents and staurosporin (14). It has also been shown that staurosporin induces apoptosis in a variety of cell lines (15). We have previously investigated the effect of protein kinase and phosphatase inhibitors on the growth of different human prostatic carcinoma cell lines and found that staurosporin inhibited the growth of both Fas-resistant and Fas-sensitive cell lines (16). In this study, we treated PC3 and DU145 with 1 µm staurosporin and investigated the time course of caspase activation using YVAD-AMC and DEVD-AMC substrates. We did not find YVADase activity under staurosporin treatment (data not shown), but DEVDase activity was induced by staurosporin treatment and was found to be time-dependent (Fig. 5). By Western blot analysis, we subsequently investigated the timing of caspase-8 and caspase-7 activation under staurosporin treatment. As shown in Fig. 6, caspase-8 was not activated by staurosporin, whereas caspase-7 was activated after 4 h of treatment with staurosporin both in the Fas-sensitive cell line PC3 and the Fas-resistant cell line DU145. These data are consistent with experiments described above, which indicate that the inhibitory protein(s) acts at the apex of the apoptotic cascade and appears to prevent the activation of caspase-8.
DISCUSSION

There are different mechanisms by which Fas-mediated apoptosis may be inhibited, and one such mechanism is the inhibition of caspase activation. Caspase activation is a crucial biochemical event involved in Fas-mediated apoptosis (1–3) and, therefore, it is of central importance to find out whether the dominant suppressor(s) of Fas-mediated apoptosis in human prostatic carcinoma cell lines, which we previously revealed (7, 8), affects the activation of caspase cascade. Caspases that participate in apoptosis are generally divided into initiators and executors (3). Signaling in Fas-mediated apoptosis occurs by direct ligation of the Fas-receptor at the cell surface, which leads to the activation of initiator caspase-8 (FLICE). Caspase-8 is recruited to the “death-inducing signal complex” that forms on the cytoplasmic portion of the Fas-receptor after Fas-receptor oligomerization, by the adaptor protein FADD/MORT1 (17–21). It has been recently shown that oligomerization of caspase-8 at the membrane (22) or clustering of procaspase-8 to the Fas/FADD complex (6) is sufficient for procaspase-8 autoactivation due to an intrinsic proteolytic activity of the zymogen. Caspase-8 then activates the executioner caspases-3, -7, or -6.

To investigate caspase involvement in Fas-mediated apoptosis in human prostatic carcinoma cell lines, we used three different approaches: (a) cleavage of two fluorogenic substrates, YVAD-AMC (ICE-like-specific substrate) and DEVD-AMC (CPP32-like-specific substrate); (b) inhibition of apoptosis by different caspase inhibitors; and (c) Western blot analysis of caspase activation using mAbs that interact both with zymogen and the large subunit of activated caspase-8, -3, and -7.

We did not find cleavage of YVAD-AMC in any of the cell lines examined. DEVD-AMC was cleaved by cell lysates obtained from Fas-sensitive cell lines PC3 and ALVA31 after Fas-ligation. This substrate was also cleaved by cell lysates from Fas-resistant cell lines DU145 and JCA1, but only when these cells were simultaneously treated with anti-Fas mAb and CHX. Thus, in human prostatic carcinoma cell lines, the executioner caspases(s) in Fas-mediated apoptosis belongs to the CPP32-like subfamily of caspases; activation of this subfamily was found to be blocked in Fas-resistant cell lines. In agreement with these results, inhibition of apoptosis by different caspase inhibitors revealed that the CPP32-like inhibitor, Z-DEVD-FMK, completely blocked apoptosis in PC3. However, complete inhibition of apoptosis in ALVA31, DU145, and JCA1 was achieved only by using the pan-caspase inhibitor Z-VAD-FMK. These data indicate that in addition to the caspase-3 subfamily, another caspase(s) might be involved in Fas-mediated apoptosis in these cell lines.

There are two major executioner caspases in the CPP32-subfamily: caspase-3 and caspase-7 (1, 2). Western blot analysis with mAbs to caspase-3 and caspase-7 revealed that only caspase-7 was activated during Fas-mediated apoptosis in all cell lines examined. We also found that only caspase-7, but not caspase-3, was activated under staurosporin treatment (Fig. 6) and under treatment with doxorubicin (data not shown). It has been recently reported that lovastatin-induced apoptosis in the human prostatic carcinoma cell line LNCaP was executed by caspase-7 (22). These data suggest that caspase-7 might be the prostate-specific executioner caspase both in Fas-mediated and drug-induced apoptosis.

To investigate whether the inhibitory protein(s) in a Fas-resistant cell line acts at the apex of caspase cascade or inhibits executioner step, we investigated the timing of caspase-8 and caspase-7 activation both in the Fas-sensitive cell line PC3 and the Fas-resistant cell line DU145. Caspase-8 was found to be activated after 1 h of Fas-ligation in PC3, but only after 4 h of treatment with anti-Fas mAb (in the presence of CHX) in DU145. The half-life time of the suppressor protein(s) in DU145 might, therefore, be estimated as <4 h. These data clearly indicate that Fas-mediated apoptosis was blocked upstream in DU145. Other evidence that the suppressor protein(s) acts at the apex of the caspase activation cascade was found in experiments with staurosporin treatment. We have found that staurosporin induced apoptosis to the same extent both in Fas-resistant and Fas-sensitive cell lines. Staurosporin-induced apoptosis is not thought to be mediated by caspase-8 activation (14). In agreement with these data, we found that under staurosporin treatment caspase-8 was not activated in both Fas-resistant and Fas-sensitive cell lines. At the same time, caspase-7 was activated in the same time-dependent manner in both PC3 and DU145.

Interestingly, we observed Rb protein degradation during Fas-mediated apoptosis in Fas-sensitive cell lines PC3 and ALVA31. Cleavage of Rb in its interior region has been described under etoposide treatment in the leukemic cell lines Jurkat and HL-60 (23). Rb protein degradation in these cell lines resulted in two fragments, p48 and p68, and proteolysis was prevented by two caspase inhibitors, YVAD-CMK and DEVD-FMK, suggesting that two caspase subfamilies are involved in Rb proteolysis. We have also found that Rb cleavage was inhibited in PC3 by YVAD-CMK and DEVD-FMK. However, Rb proteolysis in ALVA31 was prevented only by DEVD-FMK, whereas YVAD-CMK was not effective. These data, therefore, suggest that activation of CPP32-like caspases is sufficient for Rb proteolysis and, furthermore, that involvement of caspase subfamilies in Rb proteolysis might be cell-specific. To the best of our knowledge, this is the first study suggesting that Rb internal proteolysis occurs after Fas ligation and is mediated by caspases.

In conclusion, caspase-8 was found to be an initiator caspase, and we have found that caspase-7 is the executioner caspase in human prostatic carcinoma cell lines both in Fas-mediated and drug-induced apoptosis. The inhibitory protein(s), which prevents apoptosis in Fas-resistant cell lines, acts at the apex of Fas-mediated apoptosis cascade.

REFERENCES


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