Small Unstable Apoptotic Protein, an Apoptosis-associated Protein, Suppresses Proliferation of Myeloid Cells

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ABSTRACT

Apoptosis, or programmed cell death, is a process where developmental or environmental stimuli activate a genetic program to implement a series of events that culminate in cell death. To study the nature of genes that are induced during the apoptotic death of myeloid precursor cells, representational difference analysis was performed using RNAs derived from 32Dcl3 myeloblastic cells that were proliferating in the presence of IL-3 and cells that were actively undergoing apoptosis as a result of interleukin 3 deprivation for 24 h. This report describes a novel gene [small unstable apoptotic protein (SUAP)] that is up-regulated in these cells after the removal of interleukin 3 and exposure to granulocyte colony stimulating factor. The protein encoded by this gene is a target of the proteasome and does not share homology with other previously characterized proteins. To further define SUAP’s role in growth arrest and apoptosis, 32Dcl3 cells that ectopically express SUAP under the control of an inducible promoter were generated and tested for their ability to proliferate under conditions where SUAP expression is induced. These studies show that although the SUAP expressing cells exhibited suppressed proliferation rates, this was not attributable to alterations in cell cycle progression. Rather, SUAP appears to induce the appearance of Annexin V-positive cells, supporting a role for this protein in programmed cell death.

INTRODUCTION

Programmed cell death is an essential phenomenon that regulates normal development and homeostasis. In the past decade, remarkable progress has been made toward a better understanding of the molecular pathways that mediate apoptotic cell death. Apoptotic machinery is normally suppressed or activated by signals from the extracellular environment as well as intracellular sensors that monitor DNA damage. The absence of external survival signals or irreparable DNA damage are some of the important events that appear to trigger apoptosis in lower organisms such as Caenorhabditis elegans (reviewed in Ref. 1). While preserving these apoptotic responses, higher organisms such as mammals have evolved a distinctive mechanism that enables the organism to instruct certain cell populations to enter apoptotic pathways at different stages of development. This is well exemplified in the case of the immune system where it is necessary to eliminate the presence of T, B, and myeloid cell populations after their expansion in response to external infection (reviewed in Refs. 2–4). Accumulating evidence suggests that in higher organisms, apoptosis is regulated by two major pathways, one that originates at the membrane and another that involves mitochondria (reviewed in Refs. 5, 6). The apoptotic pathways that originate at the membrane involve death receptors such as Fas, tumor necrosis factor R1, DR-3, DR-4, and DR-5. These death receptors are activated by their cognate ligands resulting in the recruitment and activation of caspases, and this process does not appear to require de novo transcription and translation (5, 6). The apoptotic pathways that involve mitochondria affect mitochondrial permeability and the release of cytochrome c into the cytosol, which subsequently interacts with Apaf-1 and procaspase 9, leading to the activation of caspase 9 and the downstream caspases (reviewed in Ref. 7). In contrast to the death receptor mediated pathways, this process requires de novo mRNA and protein synthesis and involves the members of the Bcl-2 gene family. Thus, Bcl-2 and Bcl-xL inhibit the release of cytochrome c from the mitochondria and block apoptosis, whereas Bax and Bid, proapoptotic members of the family, promote the release of cytochrome c from mitochondria (5, 6, 8–11).

In the mammalian organism, hematopoietic cell growth is normally dictated by a group of growth factors known as cytokines. Recent studies have shown that cytokines not only mediate proliferation and differentiation of hematopoietic cells but also enhance the survival of these cells by the suppression of apoptotic pathways (12, 13). Withdrawal of cytokines from the culture medium has been found to result in apoptosis of hematopoietic cells, which appears to require de novo RNA and protein synthesis and has been found to involve members of the Bcl-2 gene family, suggesting the involvement of mitochondria (14–17).

To study the nature of genes that are induced or up-regulated during the apoptotic death of myeloid precursor cells, the 32Dcl3 cell line was used, which is derived from normal mouse bone marrow, and is nontumorigenic and diploid (18, 19). These cells are strictly dependent on IL-3 for growth and rapidly undergo apoptosis after deprived of this IL. To determine the nature of genes that are induced during IL-3 withdrawal-induced apoptosis of 32Dcl3 cells, RDA (20, 21) was performed using cDNAs derived from proliferating cells grown in the presence of IL-3 and cells undergoing apoptosis as a result of IL-3 deprivation. This article describes the sequence of a novel cDNA clone isolated from this study, termed SUAP, the expression of which is up-regulated in response to IL-3 withdrawal-induced apoptosis and G-CSF-induced terminal differentiation of 32Dcl3 cells. This protein product, which is unstable and subject to proteosome-mediated degradation, can suppress IL-3-dependent proliferation of 32Dcl3 cells. SUAP-mediated growth suppression is also correlated with the accumulation of Annexin V-positive cells, additionally supporting a role for this gene in cell growth and apoptosis.

 MATERIALS AND METHODS

Cell Lines. 32Dcl3 cells (18, 19) were maintained in IMDM supplemented with 10% FBS, penicillin-streptomycin, and 10% conditioned medium derived from the WEHI-3B cell line as a source of IL-3 (22). The WEHI-3B cell line was maintained in RPMI 1640 supplemented with 10% FBS and penicillin-streptomycin. The CHO-GCSF cell line (23) was maintained in IMDM supplemented with 10% FBS and penicillin-streptomycin. COS-7 cells were maintained in DMEM supplemented with 10% FBS and penicillin-streptomycin.
IL-3 Withdrawal-induced Apoptosis, G-CSF-induced Differentiation, and IL-3-induced Proliferation of 32Dcl3 Cell Lines. To induce apoptosis in 32Dcl3 cells, actively proliferating cells were washed twice in IMDM (to remove IL-3) and plated at a density of 2 x 10^7 cells/ml in IMDM supplemented with 10% FBS. Low molecular weight DNA was isolated as described previously (24). For G-CSF induced terminal differentiation, cells were treated as above except that they were plated in IMDM supplemented with 10% FBS and 10% conditioned medium derived from a CHO-GCSF cell line as a source of G-CSF. The rate of IL-3-dependent proliferation of 32Dcl3 cell lines was determined by seeding actively proliferating cells at a density of 1 x 10^5 cells/ml in IMDM supplemented with 10% FBS and 10% conditioned WEHI-3B medium as a source of IL-3. Cell number and viability were determined at daily intervals using trypan blue exclusion.

RDA, RNA Preparation, and SUAP cDNA Cloning. RDA was performed as previously described by Huhban and Schatz (21). Total RNA was isolated using the RNeasy Midi kit (Qiagen). The full-length SUAP cDNA product identified by RDA was used as a probe to screen a Agt-11 library derived from 32Dcl3 cells treated with G-CSF for 5 days (25). The three longest cDNAs were sequenced using the Sanger dideoxy method. Nucleotide sequence comparison, protein translation, and database searches were performed using MacVector software (Oxford Molecular Group).

Northern Blot Analysis. Total RNA was isolated from 32Dcl3 cells as described above. A total of 20 µg of each sample was electrophoresed on a 1% formaldehyde-agarose gel and transferred to nitro membranes (Schleicher and Schuell) for Northern blot analysis. Hybridization under stringent conditions was performed overnight and washed as described previously (26). The tissue distribution of SUAP was determined by probing a mouse multiple tissue Northern blot (Clontech) with the full-length SUAP cDNA according to the instructions of the manufacturer.

Expression Constructs and in Vivo Expression. The full-length SUAP cDNA was fused in-frame with the HA-epitope tag cDNA sequence and subcloned into the pSG5 and the pOPRSVICT expression vectors (Stratagene). For transient transfections in COS-7 cells, pSG5-SUAP/HA (5 µg) and the parental vector were introduced into actively proliferating cells by the DEAE-dextran method (27). Cells were either left untreated or treated with either MG132, lactacystin or the parental vector were introduced into actively proliferating 32Dcl3 cells by electroporation (300 V; 960 μF). Single cell clones were obtained by limiting dilution (28). To induce ectopic expression, actively proliferating cells were grown in the presence of G418 (500 µg/ml) and hygromycin (1 mg/ml). Single cell clones were obtained by limiting dilution (28). To induce ectopic expression, actively proliferating cells were grown in the presence of 5 mM IPTG for 16 h before harvesting.

Generation of a SUAP-specific Antibody. A DNA fragment encompassing the entire SUAP coding sequence was generated by PCR and subcloned into the BamHI and EcoRI sites of the pGEK-2T vector (Pharmacia) to generate a GST-SUAP fusion protein. Large scale induction was performed according to the instructions of the manufacturer. IPTG-induced bacteria were resuspended and sonicated in 5 mM urea/PBS and rotated overnight at 4 °C. The crude extract was subsequently dialyzed using serial dilutions of urea in PBS (4–0.5 M). After the protein extract was dialyzed in PBS (no urea), the remainder of the purification was performed according to the instructions of the manufacturer. The purified protein was used as an antigen to generate a rabbit polyclonal antiserum.

Flow Cytometric Analysis. Cell cycle analysis by flow cytometry was performed on a Elite Couter Counter. Cells (3 x 10^6) were washed twice with PBS, resuspended in 0.5 ml of PBS with 1% FBS, and fixed by the addition of 2 ml of ice-cold absolute ethanol while slow vortexing. After fixation, cells were washed once and resuspended in 0.8 ml of 1% FBS in PBS, 50 µl of PI (500 µg/ml in 38 mM sodium citrate) and 25 µl of 10 mg/ml RNase A and incubated at 37°C for 30 min before subjected to flow cytometric analysis.

Annexin V Staining. Annexin V staining was performed as described by the manufacturer (BD Biosciences). Briefly, 1 x 10^6 cells were washed twice in PBS and resuspended in binding buffer (10 mM HEPES, NaOH (pH 7.4), 140 mM NaCl, 2.5 mM CaCl_2) at a concentration of 1 x 10^6 cells/ml. Five µl of FITC-Annexin V (BD Biosciences) and 10 µl of PI (500 µg/ml in 38 mM sodium citrate) were added, and the cells incubated for 15 min in the dark at room temperature. A total of 400 µl of binding buffer was then added and the cells analyzed by flow cytometry.

Western Blot Analysis. Cells were lysed in 1% NP40/PBS in the presence of 1X protein inhibitor mixture (Roche) supplemented with 1 mM NaF and 1 mM Na_3VO_4. A total of 50 µg of total cell lysate was separated by SDS-PAGE (12%), transferred to a nitrocellulose membrane, and probed using commercially available antisera (Santa Cruz Biotechnology) according to the instructions of the manufacturer or the SUAP antiserum. Proteins were visualized using Renaissance Chemiluminescence Reagent (Perkin-Elmer).

RESULTS

SUAP RNA Is Up-Regulated during the Apoptotic Death of IL-3-deprived 32Dcl3 Cells. To determine the nature of genes that are induced during IL-3 withdrawal-induced apoptosis of 32Dcl3 myeloblastic cells, RDA (20, 21) was performed using poly(A)^+ mRNAs derived from actively proliferating cells grown in the presence of IL-3 and cells undergoing apoptosis as a result of IL-3 deprivation for 24 h. One clone (comprising bases 419–712 of the full-length cDNA) was used as a probe to screen a cDNA library derived from mRNA extracted from 32Dcl3 cells (25), which resulted in the isolation of an novel cDNA that predominantly hybridized to a transcript of ~1.1 kb. The nucleotide sequence and the deduced amino acid sequence encoded by the longest insert representing the full-length gene (termed SUAP) is shown in Fig. 1. An examination of this sequence revealed that this cDNA contained an initiation codon at position 192–194 and a terminator codon at position 549–551. The open reading frame consists of 357 bases, which could encode a protein of 119 amino acids of Mr = ~12,700. Although the cDNA sequence was homologous to other uncharacterized SUAP or SUAP-like sequences within the GenBank, additional searches using several

![Fig. 1. Nucleotide and deduced amino acid sequence of the murine SUAP cDNA. GenBank accession number AF592153.](attachment:image)
SUAP RNA Is Up-Regulated during IL-3 Withdrawal-induced Apoptosis of 32Dc13 Cells. 32Dc13 cells are strictly dependent upon IL-3 for proliferation (Fig. 2A) and, in the absence of IL-3, undergo rapid apoptosis within a period of 16–48 h (15, 16). Fig. 2B shows the loss of viability of 32Dc13 cells grown in IL-3-free medium. In the absence of IL-3, these cells gradually lost viability and by 24 h, ~50% of the cells were dead as measured by trypan blue exclusion. The apoptotic nature of their death was verified by monitoring the electrophoretic mobility of low molecular weight DNA, which showed a characteristic DNA ladder formation (Fig. 2C). To determine the expression of SUAP during IL-3 withdrawal-induced apoptosis of 32Dc13 cells, total RNA was isolated from cells that were deprived of IL-3 for 0, 2, 4, 16, and 24 h and subjected to Northern blot analysis. Fig. 2D shows that although basal levels of SUAP RNA are expressed in cells growing in the presence of IL-3, expression is markedly increased by 16 h upon removal of the cytokine. RNA levels remained elevated at the 24-h time point, when ~50% of the cells are dead. These results confirmed the initial results of the RDA that SUAP is up-regulated during the apoptotic death of 32Dc13 cells and suggest that this gene may act either as a positive or negative regulator of apoptosis in these cells.

Because granulocytic differentiation of hematopoietic cells is also linked to their apoptotic death (13), it was also of interest to examine the synthesis of SUAP mRNA in 32Dc13 cells after exposure to G-CSF. In the presence of IL-3, these cells proliferate as immature myeloblasts that are characterized by large nuclei that encompass >70% of the cell (Fig. 3A, day 0). In this in vitro model system, removal of IL-3 and exposure to G-CSF induces a 4–5-fold increase in cell number within 4–5 days when the cells are differentiating into myelocytes and metamyelocytes (Fig. 3A, day 2 and day 4). Cessation of proliferation occurs between days 5 and 7 and is accompanied by terminal differentiation into morphologically normal and myeloperoxidase and lactoferrin positive granulocytes (Fig. 3A, days 10, 15, and 16). Northern blot analysis of RNA extracted from these cells grown in the presence of G-CSF for 0, 2, 4, 6, 8, and 10 days is shown in Fig. 3B. As was observed in the 32Dc13 cells that had been deprived of IL-3, the SUAP probe hybridized to a 1.1-kb transcript present in G-CSF-treated cells. The synthesis of this transcript was induced in 32Dc13 cells by G-CSF and the levels of SUAP RNA reached a peak by day 6. Although expression remained stable until day 8 (a period of time when the majority of the population has terminally differentiated), the level of expression declined by day 10. These results suggest that SUAP gene transcription is up-regulated during G-CSF-induced terminal differentiation of myeloid precursor cells and support the hypothesis that SUAP expression correlates with the onset of apoptosis.

To determine if SUAP is expressed in tissues other than myeloid cells, the full-length SUAP cDNA was used to probe a murine multiple tissue Northern blot (Clontech). This result, shown in Fig. 4, demonstrates that the SUAP gene is expressed at low levels in multiple tissues, which include heart, brain, testis, liver and kidney. Expression was observed in the lung and spleen, albeit to a lesser extent.

SUAP Is a Target of the Proteasome. The SUAP amino acid sequence shown in Fig. 1 consists of 119 amino acid residues and was predicted to migrate as a Mr 13,000 protein. To determine whether the protein migrates with the predicted molecular weight, an HA-tagged SUAP cDNA was transiently transfected into COS-7 cells. Although SUAP RNA was expressed in Northern blot analysis (data not shown), significant amounts of expression could not be detected at the protein level. To address the possibility that SUAP was a target of proteasome-mediated degradation, the SUAP-transfected cells were treated with the MG132 proteasome inhibitor (reviewed in Ref. 29). Western blot analysis of protein extracts derived from these cells (Fig. 5A) shows that SUAP, which migrates as a Mr 15,000 doublet, was stabilized in a dose-dependent manner by the addition of MG132. To additionally confirm that SUAP was a target of the proteasome, a similar experiment was performed with a more specific inhibitor of the proteasome, lactacystin (29). As was observed in the MG132-treated cells, SUAP was stabilized by lactacystin, as well as by a
related compound, clasto-lactacystin-β-lactone (30), in a dose-dependent manner (Fig. 5B). The results of these studies demonstrate that the SUAP protein is unstable and is subject to degradation by the proteasome.

Ectopic Expression of SUAP Suppresses the Proliferation of 32Dcl3 Cells in Response to IL-3. To test the hypothesis that SUAP is involved in the apoptotic response of 32Dcl3 cells, an attempt was made to generate a stable cell line that constitutively expressed the SUAP protein. Because several attempts to make this cell line failed, the possibility existed that high levels of SUAP were deleterious in actively proliferating cells. Therefore, an inducible expression construct was generated that places SUAP expression under the control of an IPTG-inducible promoter using the LacSwitch system (Stratagene). This construct (and that which encodes the repressor of the IPTG-inducible promoter) was electroporated into 32Dcl3 cells and clonally derived cells that expressed high levels of SUAP (Fig. 6, A and B) were chosen for additional analysis. To determine SUAP’s effect on IL-3-mediated proliferation of 32Dcl3 cells, 32D/SUAP and vector-transfected control cells were seeded at a density of 1 × 10^5 cells/ml in the presence and absence of 5 mM IPTG. Aliquots of cells were removed at 24-h intervals, and their number and viability determined by trypan blue exclusion. The graph on the left of Fig. 6C shows that while the vector control cells proliferated normally in response to IL-3, the SUAP-transfected cells (clone 1) exhibited suppressed rates of proliferation in a SUAP-dependent manner. Although SUAP expression in response to IL-3 is tight at the level of RNA, expression at the protein level is slightly leaky in the absence of IPTG (Fig. 6, A and B). The observation that the SUAP-expressing cells grown in both the absence and presence of IPTG proliferate at a rate that is slower than the vector-transfected control indicates that SUAP-mediated growth suppression may require only a minimal amount of protein expression. To additionally confirm these results, the proliferative abilities of two additional SUAP-expressing clones
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Fig. 6. A, ectopic expression of SUAP RNA in 32D/SUAP cells. Total RNA was extracted from 32D/SUAP and control cells grown in the presence of IL-3 (and 5 mM IPTG where indicated) for 24 h and was subjected to Northern blot analysis using the full-length SUAP cDNA as a probe. 18 s and 28 s rRNA are shown as a loading control. B, inducible expression of SUAP protein in 32D/SUAP cells. 32D/SUAP and control cells were treated as described above in A. Cells were treated with 2.5 μM MG132 for 2 h before harvesting and the preparation of whole cell protein lysates. C, ectopic expression of SUAP suppresses the proliferation of 32Dcl3 cells in response to IL-3. 32D/SUAP (clones 1, 2, and 3), and control cells were seeded at a density of 1 × 10⁵ cells/ml in the presence of IL-3 (and 5 mM IPTG where indicated). Cell density and viability were determined each day using trypan blue exclusion. Three clonal cell lines are shown. These results represent those for one out of three representative experiments with similar outcome for each clonal cell line.

were analyzed by trypan blue exclusion. As can be seen in Fig. 6C, these clones (clones 2 and 3) also proliferated slower than the control cell line. Similar growth-suppressive effects were observed during G-CSF-mediated terminal differentiation of these cell lines, although no changes were observed in their cytoplasmic and nuclear morphologies (data not shown). It is therefore possible that the expression of SUAP is correlated with the growth arrest that often precedes the onset of apoptosis in 32Dcl3 cells.

SUAP Promotes Apoptosis in 32Dcl3 Cells. To determine whether the reduced proliferation observed in the 32D/SUAP cells was because of the induction of growth arrest, the kinetics of the cell cycle progression of this cell line and the vector-transfected control were analyzed by FACS analysis. Both cell lines were seeded at a density of 1 × 10⁵ cells/ml in the presence of 10% WEHI-3B-cultured supernatant (as a source of IL-3) and absence of 5 mM IPTG. The cells were harvested 48-h after plating (to avoid complications that are associated with the induction of G₁ arrest if the cells were allowed to reach saturation density before analysis) and subjected to FACS analysis using PI staining. As illustrated in Fig. 7A, the 32D/SUAP and the control cell lines demonstrated similar profiles of cell cycle distribution at the 0 and 48-h time points, and more importantly, the 32D/SUAP cells did not accumulate in the G₁-G₂ phase of the cell cycle. A second possibility remained that SUAP expression was suppressing proliferation via an induction of apoptotic pathways. To determine whether this was the case, 32D/SUAP and vector-transfected control cells were seeded as described above in the presence of IL-3. Forty-eight h after plating, the cells were harvested and subjected to staining using FITC-conjugated Annexin-V and PI to discern between viable cells, apoptotic cells with an intact membrane, and cells undergoing secondary necrosis. Fig. 7B shows that although <2% of the control cells were stained as apoptotic (high Annexin V staining, low PI staining), a larger percentage of the 32D/SUAP (7.8%) cells were stained positive for Annexin-V. Because IL-3 is a potent suppressor of apoptosis and could therefore mask the effects of SUAP expression, a similar experiment was performed using cells that have been deprived of IL-3 for a period of 24 h. The results of this experiment (Fig. 7C) show that although ~38% of the control cells were stained positive for Annexin V, a larger percentage, >50%, of the SUAP-expressing cells were stained as apoptotic. Although the number of SUAP-expressing cells that stain positive for Annexin V is similar in the presence and absence of IPTG, these results are in agreement with the growth curves presented in Fig. 6C. These observations suggest that SUAP protein is a promoter of programmed cell death in 32Dcl3 cells.

Because growth factor deprivation has been shown to promote apoptosis via the Bcl-2 family of proteins (5, 6), it was of interest to determine whether ectopic SUAP expression induced alterations in the levels of those family members that have been shown to play a role in apoptosis induced by IL-3 withdrawal (12, 15–17, 31–34). 32D/SUAP and vector-transfected cells were therefore grown in the presence and absence of IL-3 for 48 h, respectively (time points which are identical to those shown in Fig. 7, B and C), in the presence and absence of 5 mM IPTG. Protein extracts were derived from these cells and subjected to Western blot analysis using antibodies directed against the following Bcl-2 family members: Bax; Bcl-2; Bag-1; Bad; and Bcl-X (Fig. 7D). Despite the observation that a greater percentage of the SUAP-expressing cells consistently stain positive for Annexin V, no significant differences in the expression levels of these proteins could be detected by Western blot analysis.

DISCUSSION

To determine the nature of proteins that are differentially regulated as a function of apoptosis, RDA was performed using poly(A)⁺ RNAs derived from 32Dcl3 myeloblastic cells that were actively proliferating in the presence of IL-3 and 32Dcl3 cells that were actively
undergoing apoptosis as a result of IL-3 deprivation for 24 h. This report describes the isolation of a novel, uncharacterized protein, termed SUAP, the expression of which is up-regulated as a function of programmed cell death in myeloid precursor cells.

Although database searches have revealed that SUAP is homologous or identical to human and mouse expressed sequence tags and RIKEN sequences (35, 36), additional searches failed to reveal any significant homology to a particular structural motif or to a previously characterized functional domain. However, homology was observed between SUAP and two families of previously identified proteins. One family, which contains the *Drosophila melanogaster* putative zinc binding protein Yippee and its human homologue Ypel1, encodes putative zinc binding proteins that are highly conserved among eukaryotes (37, 38). Although neither protein has been shown to play a role in apoptosis, ectopic expression of Ypel1 in fibroblasts induces an epithelial-like morphology that is accompanied by changes in the cytoskeleton and cellular adhesion properties (38), indicating that these proteins may regulate cellular proliferation and/or differentiation. SUAP is also homologous to genes that are related to those that have been implicated in DiGeorge/CATCH22 syndrome, a syndrome that is characterized by hypocalcemia, cardiac defects, and a hypoplastic thymus (reviewed in Ref. 39).

Because high levels of SUAP protein can only be detected in cells that have been treated with proteasome inhibitors, it is likely that SUAP is a target of the proteasome and is subject to degradation via ubiquitination (reviewed in Ref. 40). SUAP contains nine lysine residues that are scattered throughout the entire length of the protein, and it is therefore possible that one or more of these lysine residues represents a site(s) for the conjugation of ubiquitin and subsequent degradation by the proteasome. It is also of interest to note that one of these lysine residues, Lys89, closely conforms to a consensus sumoylation site (41, 42). Although SUMO modification does not regulate protein stability directly, it does control subcellular localization that can indirectly influence protein stability (43, 44). Many proteins such as members of the p53 family are both ubiquitinated and sumoylated and both modifications have been shown to influence the ability of these proteins to induce growth arrest and apoptosis (41, 45, 46).

In the normal tissues that were examined and in 32Dcl3 cells that are actively proliferating in the presence of IL-3, the 1.1-kb SUAP message is expressed at basal levels. However, removal of IL-3 from 32Dcl3 cells, which has been shown to induce rapid apoptosis (15, 16), resulted in a dramatic up-regulation of the SUAP message. Furthermore, treatment of 32Dcl3 cells with G-CSF induces terminal differentiation of these cells into mature neutrophilic granulocytes that is followed by cell death, also resulted in up-regulation of the SUAP message. Because terminal differentiation in hematopoietic cells and this model system is linked to apoptosis (13), both results are suggestive of a role for SUAP in the programmed cell death of myeloid cells.

32Dcl3 cell lines that constitutively expressed SUAP were unobtainable, indicating that SUAP expression was detrimental to actively dividing cells. Therefore, to determine whether SUAP plays a role in the proliferation and apoptosis of 32Dcl3 cells, clonally derived cells were generated that ectopically expressed the SUAP protein under the control of an IPTG-inducible promoter. When these cells were grown presence of IL-3 for 48 h were stained with FITC-Annexin V and PI and subjected to FACS analysis. The percentage of Annexin V-positive cells indicative of apoptosis (high Annexin V/low PI staining) are depicted in a graphical representation. Results represent the average of three independent experiments. C, same as for B, except that both cell lines were grown in the absence of IL-3 for a period of 24 h. D, expression of Bcl-2 family members in SUAP-expressing (clone 1) and vector-transfected control cells in the presence and absence of IL-3.

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Fig. 7. A, cell cycle analysis of 32D/SUAP cells grown in the presence of IL-3. A total of 3 x 10^7 32D/SUAP (clone 1) and vector-transfected 32Dcl3 cells were exponentially cultured in IMDM supplemented with 10% WEHI-3B-cultured supernatant for 48 h, and the percentage of cells in the G0-G1, S, and G2-M phases were determined by FACS analysis after staining with PI. B, ectopic SUAP expression increases the percentage of Annexin V-positive cells. 32D/SUAP (clone 1) and vector-transfected cells grown in the presence and absence of IL-3 were stained with FITC-Annexin V and PI and subjected to FACS analysis. The percentage of Annexin V-positive cells indicative of apoptosis (high Annexin V/low PI staining) are depicted in a graphical representation. Results represent the average of three independent experiments. C, same as for B, except that both cell lines were grown in the absence of IL-3 for a period of 24 h. D, expression of Bcl-2 family members in SUAP-expressing (clone 1) and vector-transfected control cells in the presence and absence of IL-3.
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REFERENCES


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