Suppression of Apoptosis in Hematopoietic Factor-Dependent Progenitor Cell Lines by Expression of the FAC Gene

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Fanconi anemia (FA) is a genetically heterogeneous, inherited blood disorder characterized by bone marrow failure, congenital malformations, and a predisposition to leukemias. Because FA cells are hypersensitive to DNA cross-linking agents and have chromosomal instability, FA has been viewed as a disorder of DNA repair. However, the exact cellular defect in FA cells has not been identified. Sequence analysis of the gene defective in group C patients (FAC) has shown no significant homologies to other known genes. The FAC protein has been localized to the cytoplasm, indicating that FAC may either play an indirect role in DNA repair or is involved in a different cellular pathway. Recent evidence has indicated that FA cells may be predisposed to apoptosis, especially after treatment with DNA cross-linking agents. The demonstration that genes can suppress apoptosis has been accomplished by overexpression of such genes in growth factor-dependent cell lines that die by apoptosis after factor withdrawal. Using retroviral-mediated gene transfer, we present evidence that expression of FAC in the hematopoietic factor-dependent progenitor cell lines 32D and MO7e can suppress apoptosis induced by growth factor withdrawal. Flow cytometry and morphologic analysis of propidium iodide stained cells showed significantly lower levels of apoptosis in FAC-retroviral transduced cells after growth factor deprivation. Expression of FAC in both cell lines promoted increased viability rather than proliferation, which is consistent with other apoptosis-inhibiting genes such as Bcl-2. These findings imply that FAC may act as a mediator of an apoptotic pathway initiated by growth factor withdrawal. Furthermore, the congenital malformations and hematologic abnormalities characterizing FA may be related to an increased predisposition of FA progenitor cells to undergo apoptosis, particularly in the absence of extracellular signals.

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Aptosis or programmed cell death is known to be involved in many biologic processes, including embryonic development and hematopoiesis. Cells undergoing apoptosis exhibit a series of morphologic features, including a decrease in cell size, chromatin condensation, blebbing of the plasma membrane, and cleavage of DNA at internucleosomal sites. Apoptosis has been implicated in many aspects of embryogenesis, including formation of the forelimb, facial processes, and neural development. Withdrawal of extracellular signals can induce apoptosis in many fetal and adult tissues. Growth factors and cytokines are considered to be key regulators of hematopoiesis, mainly by stimulating growth or maintaining viability of progenitor cells. For example, hematopoietic precursors undergo apoptosis upon withdrawal of growth factors. Although the diverse phenotypes of FA appear unrelated, the defects in FA cells may be explained by an increased predisposition towards apoptosis, particularly in the absence of extracellular signals. Indeed, several reports have shown that cultured FA lymphoblasts produce a reduced level of interleukin-6 (IL-6) compared with normal lymphoblasts and that addition of IL-6 to the culture medium reduces the sensitivity of FA cells to mitomycin C (MMC) and diepoxybutane (DEB) and decreases the number of chromosomal breaks. IL-6 has been shown to suppress apoptosis induced by cytokine deprivation or cytotoxic drug exposure in plasma cells.

Recent evidence suggests that the FAC protein may play a role in the survival of hematopoietic cells. Antisense oligodeoxynucleotides (ODN) complementary to FAC mRNA inhibited the in vitro clonal growth of normal erythroid and granulocyte-macrophage progenitor cells, even in the presence of exogenous growth factors. In contrast, peripheral blood CD34+ cells isolated from an FA-C patient and infected with a recombinant adenovirus expressing FAC cDNA exhibited a marked increase (5- to 10-fold) in viability.
the number of progenitor colonies formed in vitro. These results suggest that the FAC gene may play a direct role in the survival or viability of hematopoietic progenitor cells.

Previous studies showing the ability of genes to suppress apoptosis, as in the case of Bel-2 and Bel-x1, have been accomplished by overexpressing such genes in factor-dependent hematopoietic cell lines.19-21 These experiments showed the potential of a gene to prevent or delay apoptosis after withdrawal of the growth factor. 32D is a myeloid cell line established from a murine long-term bone marrow culture and is dependent on IL-3 for survival.22-23 MO7e is a human cell line derived from a megakaryocytic leukemia and is dependent on either IL-3 or granulocyte-macrophage colony-stimulating factor for survival.24-25 Both cell lines die by apoptosis if deprived of IL-3. We report that high expression of the FAC gene in 32D and MO7e cells using retroviral gene transfer delayed the onset of apoptosis in both cell lines after IL-3 withdrawal, without the abolishment of long-term factor dependence.

MATERIALS AND METHODS

Cell lines and culture conditions. The immature murine myeloid cell 32D.C1 is a subclone generated from the 32D.C13 cell line26 and was a gift from L. Penn (The Hospital for Sick Children, Toronto, Ontario, Canada). This cell line was grown in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum (FCS) and 1% conditioned media from X63Ag-653 cells (which have been transfected with a murine IL-3 cDNA vector and, thus, constitutively secrete IL-3).27 The human cell line, MO7e,23 is a subline of MO7 cells originally isolated from a patient with acute megakaryocytic leukemia28 and was a gift from J. Dick (The Hospital for Sick Children, Toronto, Ontario, Canada). This cell line was maintained in α-minimal essential medium (α-MEM) supplemented with 10% FCS and 10 U/mL recombinant IL-3 (Amgen, Ontario, Canada). The retroviral packaging cell line PA317 was grown in α-MEM media supplemented with 10% FCS.

Infection and subcloning of 32D cells. The FAC retroviral vector used to infect 32D cells contains a 1.7-kb human FAC cDNA insert under the control of the Moloney murine leukemia virus (MoMuLV) 5' LTR linked in tandem with a SV40 origin of differentiation Neo gene.29 The FAC retroviral vector used to infect MO7e cells is based on the pBabe-Neo vector, is a previously described FAC retroviral vector.30 The FAC cDNA insert contains a 2.4-kb FAC cDNA insert under the control of the SV40 enhancer and promoter. The FAC cDNA insert was obtained by digesting the pFAC3 plasmid with SmaI and then subcloning this fragment into the SphI site of pBabe-Neo. Both vectors as well as pBabe-Neo were transfected into the amphotropic packaging cell line PA317 for production of retroviral particles. For infection of 32D cells, packaging cell lines were grown to near confluence and the culture supernatants were kept for further studies. Western blotting analysis. Cells were washed once with cold phosphate-buffered saline (PBS) and lysed by the addition of lysis buffer (50 mmol/L Tris-HCl [pH 7.5], 150 mmol/L NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], and 0.2 mmol/L phenylmethylsulfonyl fluoride). Insoluble material was removed by centrifugation at 10,000 g for 2 minutes at 4°C. Protein concentrations were determined by using the Bradford reagent as recommended by the manufacturer (Bio-Rad, Richmond, CA). After normalization for protein content, 20 μg of each sample was separated in reducing conditions in a 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel and electroblotted onto Immobilon-P membranes (Millipore, Bedford, MA). After blockage for 2 hours at normal temperature in a solution of TBS containing 5% milk and 0.5% Tween-20, membranes were incubated with the primary antibody in blocking buffer for an additional 2 hours. The affinity-purified FAC polyclonal antibody, which was made against a recombinant FAC fusion protein,23 was used at a concentration of 0.6 μg/mL. After washing, blots were probed with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (Amersham, Arlington Heights, IL) at a dilution of 1:10,000, followed by further washing and detection using the enhanced chemiluminescence detection system (Amersham). Cell proliferation assay. Cell proliferation was measured by suspending 1×10⁶ cells in 24-well plates in triplicate 1-mL cultures. Plates were incubated at 37°C in the absence of growth factor. After the indicated time, cultures were pulsed with 1 μCi of [3H]thymidine (Amersham) and harvested 14 to 16 hours later on glass fiber filters. Results were expressed as the mean cpm ± standard deviation (SD) of triplicate cultures.

RESULTS

Retroviral-mediated transfer of the FAC gene into 32D and MO7e cells. Transfer of the FAC gene into 32D cells was accomplished using the supernatant from a cell line producing a previously described FAC retroviral vector. The Neomycin retroviral vector used as a control contained only the neomycin resistance gene under the control of the SV-40 promoter. Both vectors were previously transfected into the packaging cell line PA317 and generated titers ranging from 1 to 3×10⁸ infectious particles/mL. To minimize heterogeneity, transduced 32D cells were subcloned by selection in methicillin containing G418 (1 mg/mL). MO7e cells were infected with a retroviral vector similar to
Western blot analysis of FAC expression in parental and FAC-transduced cell lines. (A) FAC expression in 32D cells and two FAC subclones D2 and D5 in the presence of IL-3 (+) and after factor deprivation for the indicated times. Strong expression of 60-kD and 55-kD FAC protein products was seen only in the FAC subclones with no detectable expression in the parental cell line. (B) Expression of a 60-kD FAC protein in MO7e and MO7e-FAC cells before (+) and after IL-3 withdrawal. Only MO7e-FAC cells constitutively expressed FAC after factor deprivation. All immunoblots were probed with an anti-FAC polyclonal antibody (0.6 μg/mL).

The vector used to infect 32D cells except that it contained a 2.4-kb FAC cDNA insert composed of the FAC reading frame and portions of the FAC 5' and 3' UTR. After viral infection, MO7e cells were grown in suspension and selected by neomycin resistance for 10 days. Because of the poor clonality of MO7e cells, bulk populations of infected cells, MO7e-Neo and MO7e-FAC, were used in all subsequent experiments.

To ensure correct viral integration, genomic DNA from transduced cell lines was extracted and digested with restriction enzymes that cleaved exclusively in both the 5' and 3' LTR. Southern analysis using an FAC-specific probe showed a 4.6-kb and a 7.0-kb band in 32D and MO7e cells, respectively, only in FAC-transduced cells, indicating an unrearranged integrated proviral genome (data not shown). Western blotting was performed to determine if FAC-transduced cells produced increased amounts of FAC protein in the presence of IL-3 (Fig 1). In addition, protein extracts of the parental and transduced cell lines were examined at various times after IL-3 deprivation. A rabbit polyclonal antibody produced against an FAC bacterial expression product, which recognizes both human and mouse FAC protein, was used to detect the retrovirally derived and endogenous FAC protein. The parental 32D cell line produced no detectable endogenous Fac protein in the presence or absence of IL-3, whereas the 32D-FAC clones produced abundant amounts of both a 60- and 55-kD protein product (Fig 1A). The 60-kD protein conforms to the previously recognized molecular weight of FAC, whereas the 55-kD product may represent a smaller product derived from a potential downstream in-frame translational start site. MO7e-FAC cells had similar levels of the 60-kD FAC protein as the parental cell line (Fig 1B). However, in this case, the protein was constitutively expressed after IL-3 withdrawal, whereas the endogenous levels of FAC in the parental cell line decreased until no detectable levels of FAC were seen past the 72-hour time point.

**FAC-retroviral transduced cells have increased viability after IL-3 withdrawal.** To examine the effect of FAC expression on cell viability after IL-3 withdrawal, control cells and FAC-transduced cells were grown logarithmically for several days and then were thoroughly washed and resuspended in media lacking IL-3 in replicates of three. At 24-hour periods afterwards, cell viability was determined by trypan blue dye exclusion. 32D clones overexpressing FAC showed increased viability compared with Neo controls (Fig 2A). After 2 days, IL-3-deprived FAC clones had between 40% and 60% cell survival, whereas Neo clones had less than 5% viability. Unlike 32D-Neo cells, the majority of MO7e-Neo cells did not die within the first 48 hours, but rather after 48 hours. After factor deprivation, MO7e-FAC cells had dramatically higher viability than MO7e-Neo cells (Fig 2B). MO7e cells constitutively expressing FAC had 75% viability up to 4 days after IL-3 withdrawal, whereas
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Fig 2. Retroviral-mediated FAC expression enhances viability without affecting cellular proliferation after IL-3 withdrawal. Survival of 32D (A) and M07e (B) cells, transduced with either Neo or FAC retroviral vectors, after IL-3 deprivation. Cells were thoroughly washed in PBS and resuspended in media lacking IL-3. Viability was determined at the indicated time points by trypan blue exclusion. The percentage of cell survival was determined by dividing the number of viable cells per milliliter by the initial cell concentration. Data are expressed as the mean ± SD of triplicate cultures.

MO7e-Neo cells had less than 20% viability. Three independent pools of Neo and FAC-transduced MO7e cells were analyzed for increased survival after IL-3 withdrawal, with minimal variability seen between pools (data not shown).

To determine if the increased survival of FAC-transduced cells was due to an enhancement of cellular proliferation, [3H]thymidine incorporation was monitored after IL-3 starvation. Both 32D cells overexpressing FAC and mock-infected cells displayed similar decreasing levels of [3H]thymidine incorporation (Fig 2C). MO7e-Neo and MO7e-FAC cells also showed similar decreasing levels of [3H]thymidine uptake after IL-3 withdrawal in 32D (C) and MO7e (D) cells infected with either Neo or FAC retroviral vectors. A total of 1 x 10^6 cells was seeded per well at time 0. Data are expressed as the mean ± SD of triplicate cultures.

The increased viability of FAC-transduced cells after IL-3 withdrawal is due to a delay in the onset of apoptosis. To determine if the increased viability of FAC-transduced cells, after IL-3 deprivation, was due to a suppression of apoptosis, flow cytometric analysis of propidium iodide-stained cells was performed. Apoptotic cells exhibited less propidium iodide fluorescence than did viable cells, owing to chromatin condensation and DNA cleavage, resulting in a subdiploid population. After IL-3 withdrawal, all cells showed G1 arrest, as indicated by the strong peak at a fluorescence intensity of 350 (Fig 3). 32D cells expressing high levels of FAC showed significantly fewer apoptotic cells than the Neo control, as shown by a comparison of the subdiploid peaks (Fig 3A). At 24 hours after IL-3 withdrawal, 32D-FAC cells had fewer than 10% apoptotic cells, whereas 32D-Neo cells had levels of apoptosis ranging from 25% to 40%. Similarly, at 48 hours, FAC clones had approximately 50% fewer apoptotic cells than Neo clones. MO7e-Neo cells did not show significant levels of apoptosis until 48 hours after IL-3 withdrawal (Fig 3B). At this time, approximately 50% of MO7e-Neo cells were apoptotic, whereas 25% of MO7e-FAC cells displayed apoptotic fragmented DNA. At 72 hours of IL-3 deprivation, a similar 50% reduction in apoptotic cells was seen in the FAC-transduced cells compared with the Neo control.

Propidium iodide-stained 32D cells were also analyzed by fluorescence microscopy. Cells displaying characteristic features of apoptosis, such as condensed chromatin and subnuclear bodies, constituted approximately 65% ± 5% of the 32D-Neo clones deprived of IL-3 for 24 hours, whereas significantly fewer (33% ± 11%, P < .02) apoptotic cells...
Fig 3. DNA fluorescence histograms of propidium iodide-stained FAC-transduced cells after cytokine deprivation. 32D (A) and MO7e (B) cells (1 x 10^6 cells in total), transduced with either Neo or FAC retrovial vectors, were analyzed in each histogram. The percentage of apoptotic cells (subdiploid peak) is indicated in brackets.
were observed in parallel cultures of 32D-FAC clones (Fig 4).

To further show that 32D-FAC clones had reduced levels of apoptosis, gel electrophoresis of genomic DNA was performed. Apoptosis typically results in degradation of nuclear DNA into fragments of integral multiples of the nucleosomal distances (≈180 bp). Lysates of cells deprived of IL-3 for 18 hours were resolved by agarose gel electrophoresis and then stained with ethidium bromide. 32D-Neo clones had marked DNA fragmentation resulting in the characteristic DNA laddering pattern indicative of apoptosis (Fig 5). In contrast, 32D-FAC clones showed little apoptotic DNA fragmentation.

**DISCUSSION**

Elucidation of the basic cellular defect in FA has been difficult. Although FA has traditionally been grouped with inherited chromosomal instability syndromes, such as ataxia telangiectasia (AT), xeroderma pigmentosum (XP), and Bloom syndrome (BS), FA is unique in that the disease causes specific congenital malformations and severe hematopoietic defects. Analysis of both the FAC nucleotide and amino acid sequences has provided no clues about the function of this gene. Furthermore, the FAC protein has been localized to the cytoplasm, indicating that FAC is unlikely to play a direct role in DNA repair or chromosomal stability. The data presented here suggest that the FAC gene is
The amino terminal truncated protein produced in patients with the delG322 mutation can partially correct the MMC sensitivity of FA-C lymphoblasts. Surprisingly, the 55-kD FAC protein produced in 32D-FAC cells is more abundant both in the presence and absence of IL-3. It would be of interest to isolate the smaller protein and determine if this product alone can suppress factor deprivation-induced apoptosis.

The amount of FAC protein in 32D-FAC cells appears to be correlated with the degree of inhibition of apoptosis. The parental cell line 32D expressed no detectable FAC and displayed marked levels of apoptosis by 24 hours (Fig 3A). The amount of FAC protein in both the D2 and D5 clones was abundant in the presence of IL-3 (Fig 1A). However, after factor deprivation, FAC levels decreased until only minimal levels of FAC were apparent at 48 hours. A significant reduction in cell viability was observed at this time point in FAC-transduced 32D cells (Fig 2A), with an increase in apoptotic death (Fig 3A). Given that the degree of identity between the human and mouse protein is 67%,21 and that cross-species functionality may be only partially preserved, it is possible that expression of the human protein in 32D cells results in incomplete suppression of apoptosis, whereas expression of the mouse homologue may have a more dramatic effect.

Retroviral-mediated expression of FAC in M07e cells did not result in a significant increase of FAC protein above endogenous levels (Fig 1B). Using in vitro protein expression studies in different human cell lines, we have found that expression of wild-type FAC via both retroviral and episomal vectors results in similar or only slightly higher levels of FAC protein when compared with its endogenous counterpart. Posttranslational mechanisms may prevent expression of high FAC levels in certain cell types. Nevertheless, when comparing FAC expression in M07e-FAC cells and the parental cell line, M07e-FAC cells constitutively express FAC after IL-3 withdrawal, whereas M07e cells display a marked reduction of protein until little or no FAC is detectable at 120 hours. Once again, the level of FAC is inversely related to the level of apoptosis. In the presence of IL-3, both M07e and M07e-FAC cells have approximately equal levels of FAC. However, after IL-3 withdrawal, M07e cells have a significant reduction in FAC levels by 48 hours, whereas minimal reduction is seen in M07e-FAC cells. Comparing cell survival at 48 hours in Fig 2, M07e-Neo cells had 50% viability whereas M07e-FAC cells had 90% viability. The increased survival of FAC transduced cells at 48 hours was related to a suppression of apoptosis because levels of apoptotic DNA were 50% in mock-infected cells, whereas only 25% in M07e-FAC cells (Fig 3B). The trend of constitutive FAC expression and suppression of apoptosis

Fig 5. 32D-FAC cells have reduced levels of internucleosomal DNA cleavage after IL-3 withdrawal. 32DNeo and 32-FAC cells were deprived of IL-3 for 18 hours, and then cell lysates were analyzed by agarose gel electrophoresis. Internucleosomal cleavage results in the appearance of 200-bp incremental DNA fragments (DNA laddering), a hallmark of apoptosis. Lane 1, 1-kb DNA ladder; lanes 2 and 3, clones Neo A3 and FAC D2 growing logarithmically in the presence of IL-3; lanes 4 through 7, clones Neo A3 and FAC D2, FAC D5, and FAC E1, respectively, in the absence of IL-3.

important in regulating the apoptotic pathway initiated in hematopoietic cells in response to growth factor deprivation.

Initial examination of FAC expression using polymerase chain reaction and reverse-transcribed RNA showed ubiquitous low-level expression in all adult human tissues examined. However, RNA in situ hybridization analysis of developing mouse embryos detected high levels of Fac transcripts in osteogenic progenitor cells in the perichondrial layer of developing bones, in the mesenchyme of the spinal cord, and in the ependymal cell layer of the developing brain. High levels of Fac transcripts have also been detected in early pluripotent hematopoietic progenitor cells in a pattern similar to that of bcl-2. Thus, upregulation of Fac expression in a specific subset of cells appears to play a role in embryogenesis and hematopoietic development.

Overexpression of FAC cDNA appears necessary to delay the onset of apoptosis in 32D cells. Interestingly, the parental 32D cell line expressed no detectable FAC protein. Retrovirally mediated expression of FAC resulted in the production of two protein products. The 60-kD product corresponds to the full-length 558 amino acid protein, whereas the 55-kD product is either a cleavage product or a smaller protein resulting from an internal translation initiation at either methionine 48 or methionine 55. The latter hypothesis is favored because in vitro transcription-translation experiments showed both 60-kD and 55-kD bands. Recently, it has been shown that FA patients with a mild form of the disease have a nonsense mutation in exon 1 (delG322) and have reinitiation of translation at methionine 55.26 The amino-terminal truncated protein produced in patients with the delG322 mutation can partially correct the MMC sensitivity of FA-C lymphoblasts. Surprisingly, the 55-kD FAC protein produced in 32D-FAC cells is more abundant both in the presence and absence of IL-3. It would be of interest to isolate the smaller protein and determine if this product alone can suppress factor deprivation-induced apoptosis.

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continues up to 120 hours after factor deprivation in M07e-FAC cells. 32D cells may die more readily than M07e cells after IL-3 starvation, due to the fact that they express little or no endogenous Fac, whereas M07e cells express abundant amounts under normal growth conditions.

Although FAC expression suppressed the early onset of apoptosis in both 32D and M07e cell lines, this suppression was not indefinite and long-term factor-dependence was not abolished. Both 32D-FAC and M07e-FAC cell lines stopped proliferating after IL-3 withdrawal (Fig 2C and D). Thus, FAC expression promoted increased viability rather than proliferation.

The ability of FAC to delay the onset of apoptosis in factor-deprived cells closely resembles that of Bcl-2. The Bcl-2 gene encodes a 26-kD protein that localizes to mitochondria, endoplasmic reticulum, and nuclear membranes and is believed to suppress apoptosis via an antioxidant pathway. Overexpression of Bcl-2 in IL-3–dependent cell lines has been shown to delay the onset of apoptosis after IL-3 deprivation, without leading to factor-independence. Comparison of the graph of 32D cells overexpressing FAC (Fig 2A) with graphs of 32D cells expressing high levels of Bcl-2 shows similar profiles after IL-3 deprivation. Thus, FAC can inhibit apoptosis in factor-deprived 32D cells at a level comparable to that of Bcl-2.

Given that FAC and Bcl-2 can similarly suppress apoptosis in 32D cells, it is possible that FAC may act by altering Bcl-2 expression. To evaluate this hypothesis, Bcl-2 protein expression was examined in both cell lines before and after IL-3 deprivation. No change in the BCL-2 protein expression pattern was seen in either Neo or FAC-transduced cells, both in the presence or absence of IL-3 (data not shown). Thus, FAC expression does not upregulate BCL-2 protein levels.

Artificially overexpressing FAC in hematopoietic precursor cell lines and preventing apoptosis may mimic the physiologic role this gene plays in maintaining the viability of bone marrow precursors. Overexpression of FAC, using a recombinant adeno-associated virus in hematopoietic progenitor cells isolated from an FA-C patient, resulted in a dramatic increase in the growth potential of these cells. Conversely, inhibition of FAC expression, using antisense oligodeoxynucleotides complementary to FAC mRNA, inhibited the in vitro clonal growth of bone marrow cells and hastened apoptotic death in factor-deprived M07e cells.

Recently, it has been shown that FA lymphoblasts die by apoptosis in response to MMC treatment and that expression of FAC in FA-C lymphoblasts can alleviate the MMC hypersensitivity of these cells and their predisposition to undergo apoptosis. However, these experiments did not determine if the primary defect of FA cells is an increased general predisposition towards apoptosis or whether the increased apoptosis reflects the fact that FA cells are simply more sensitive to MMC. If the latter were the case, exposure of FA-C and wild-type cells to equitoxic doses should lead to similar levels of apoptosis, and it could be argued that the principal defect in FA cells is an inability to repair DNA damage or detoxify DNA-damaging agents while apoptosis occurs as a consequence of these primary defects. In contrast to the experiments using MMC, the evidence presented here shows that expression of FAC in both 32D and M07e cells delays the onset of apoptosis after IL-3 deprivation, independent of an FA phenotype. Therefore, FAC appears to act directly in an apoptotic pathway initiated in response to a normal physiologic mechanism, ie, factor deprivation, as opposed to apoptosis initiated by drug-induced DNA damage. Apoptosis plays an important role in hematopoietic homeostasis by ensuring that the rate of new cell production in self-renewing progenitor cells is offset by a commensurate rate of cell death.

Given its pattern of expression in vivo, it is possible that FAC has two roles: the prevention or regulation of an apoptotic pathway during embryogenesis and hematopoiesis, which requires high levels of expression, and a housekeeping role in response to an apoptotic pathway induced by DNA damage, which requires low-level expression. Identification of proteins that interact with the FAC protein, particularly during apoptosis induced by growth factor withdrawal or DNA damaging agents, should aid in showing the mechanism defective in FA cells.

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