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Forced Expression of the Interferon Regulatory Factor 2 Oncoprotein Causes Polyploidy and Cell Death in FDC-P1 Myeloid Hematopoietic Progenitor Cells[1]

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ABSTRACT

The IFN regulatory factor-2 (IRF-2) oncoprotein controls the cell cycle-dependent expression of *histone H4* genes during S phase and may function as a component of an E2F-independent mechanism to regulate cell growth. To investigate the role of IRF-2 in control of cell proliferation, we have constructed a stable FDC-P1 cell line (F2) in which expression of IRF-2 is doxycycline (DOX)-inducible, and a control cell line (F0). Both the F2 and F0 cell lines were synchronized in the G_1 phase by isoleucine deprivation, and IRF-2 was induced by DOX on release of cells from the cell cycle block. Flow cytometric analyses indicated that forced expression of IRF-2 has limited effects on cell cycle progression before the first mitosis. However, continued cell growth in the presence of elevated IRF-2 levels results in polyploidy (>4*n*) or genomic disintegration (<2*n*) and cell death. Western blot analyses revealed that the levels of the cell cycle regulatory proteins cyclin B1 and the cyclin-dependent kinase (CDK)-inhibitory protein p27 are selectively increased. These changes occur concomitant with a significant elevation in the levels of the FAS-L protein, which is the ligand of the FAS (Apo1/CD95) receptor. We also found a subtle change in the ratio of the apoptosis-promoting Bax protein and the antiapoptotic Bcl-2 protein. Hence, IRF-2 induces a cell death response involving the Fas/FasL apoptotic pathway in FDC-P1 cells. Our data suggest that the IRF-2 oncoprotein regulates a critical cell cycle checkpoint that controls progression through G_2 and mitosis in FDC-P1 hematopoietic progenitor cells.

INTRODUCTION

IRF-2[3] is a transcription factor with an important physiological role in cell growth regulation and IFN responsiveness (1–4). Our laboratory initially identified IRF-2 [also known as Histone Nuclear Factor-M (HiNF-M)]) as a transcription factor that interacts with a phylogenetically conserved cell cycle regulatory element in the promoter of a DNA replication-dependent human *histone H4* gene (designated *FO108*, *H4/n*, or *H4FN*) that is up-regulated at the G_1-S phase transition in conjunction with DNA synthesis (5–13). IRF-2 is a repressor of IRF-1-dependent gene activation (1–4, 14–16). However, other studies have revealed that the protein is a modest activator of several genes, including *histone H4* (5, 6), *vascular cell adhesion molecule-1* (*VCAM-1*) (17), and *gp91phox* (18). IRF-2 contains a latent activation domain (19) and is a member of the winged helix-turn-helix family of transcription factors (20, 21). The activity of IRF-2 can be modulated by phosphorylation, acetylation, or interaction with coregulatory factors (22–25). The protein regulates transcription of its target genes at least in part by heterodimerization with other IRFs, and by influencing the activity of large protein-DNA complexes (enhanceosomes; Refs. 6, 26–29).

Many genes up-regulated in late G_1 are involved in DNA synthesis and nucleotide metabolism and are controlled, at least in part, by the E2F class of transcription factors (30, 31). However, DNA replication-dependent histone genes represent an important exception to this rule. The majority of histone genes are transcriptionally controlled at the G_1-S phase transition and lack functional E2F binding sites (32, 33). Our previous studies have shown that cell cycle-controlled *histone H4* genes represent physiological and S phase-specific target genes for IRF factors (5, 6). Genetic ablation of IRF-2 abrogates the cell cycle-dependent up-regulation of *histone H4* gene expression (10). One question that arises is whether the IRF-dependent regulation of histone genes reflects broader participation of IRF factors in cell cycle control. We propose that IRF factors may regulate cell proliferation through transcriptional mechanisms that operate parallel to, and/or independent of, E2F proteins.

Several studies have shown that IRF-2 has properties of an oncoprotein. For example, IRF-2 can influence cell proliferation both in culture and *in vivo* and is capable of controlling cell cycle-regulatory proteins as well as DNA-replication-dependent histone genes (5, 34–39). IRF-2 also plays a key role in cell growth and differentiation of lymphocytes, and IRF-2 deficiency compromises development of natural killer and T helper type 1 cells (40–42). We have previously analyzed the regulation of IRF-2 and other histone gene transcription factors during the cell cycle in FDC-P1 myeloid progenitor cells to understand the role of IRF-2/HiNF-M in transcriptional control of gene expression at the G_1-S-phase transition (5, 43, 44). In this study, we directly tested the role of IRF-2 in regulating cell cycle progression by conditionally expressing IRF-2 in stable FDC-P1 cell lines. The principal result of our study is that forced expression of IRF-2 results in the abrogation of stringent cell growth control, reflected by the formation of polyploid cells and cell death. Our findings suggest that balanced physiological levels of IRF-2 are critical for normal cell growth regulation.

MATERIALS AND METHODS

Construction of a DOX-inducible IRF-2 Expression Vector. We prepared a conditional expression vector based on pRetroON (Clontech Laboratories, Inc., Palo Alto, CA) by cloning the full-length human IRF-2 coding sequence into the *Bam*HI restriction endonuclease site. The IRF-2 expression vector was derived from pcDNA/IRF-2 (2, 5); this vector contains a 1.4-kbp DNA fragment spanning the human IRF-2 coding sequence that was removed by *Bam*HI cleavage (New England Biolabs, Beverly, MA). The insert in the pRetroON/IRF-2 construct was subject to automated DNA sequencing (ABI model 377) to verify the start of the open reading frame and the orientation of the cDNA insert.

Cell Culture and Stable Transfection Experiments. Actively proliferating cultures of FDC-P1 cells (45) were maintained in RPMI 1640 medium with supplements (1% of IL-3-conditioned medium, 10% FCS, 100 units/ml penicillin,100 μg/ml streptomycin, and 0.2 μM L-glutamine), at 37°C in humidified air containing 5% CO_2. RPMI 1640 and FCS were obtained from Life Technologies, Inc. (Grand Island, NY). Penicillin, streptomycin, and L-glutamine were purchased from Sigma Chemical Co. (St. Louis, MO). IL-3-

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[3] The abbreviations used are: IRF, IFN regulatory factor; DOX, doxycycline; IL, interleukin; FACS, fluorescence-activated cell sorting; CDK, cyclin-dependent kinase; FasL, Fas ligand.

conditioned medium was derived from WEHI-3B cells that produce and secrete IL-3 (46). The optimal amount of IL-3 for cytokine-dependent growth of FDC-P1 cells was empirically determined.

Experiments in which the DOX-inducible IRF-2 expression vector was transiently introduced into FDC-P1 cells were performed using the calcium phosphate-DNA precipitation method. Stable transfections with FDC-P1 cells were performed with 0.5 to 1.0 μg of pRetroON/IRF-2 expression construct or empty vector containing the puromycin gene as a positive selectable marker. Both plasmids were added into 0.15 ml of a 0.25 M $CaCl_2$ solution in a 1.5-ml sterile tube, and the solution was gently mixed. To this solution, we slowly added 0.15 ml of 2× HEPES buffer (pH 7.11) and the $CaCl_2$/DNA mixture was allowed to precipitate for 10 to 15 min at room temperature. FDC-P1 cells ($2–3 \times 10^6$ cells) were collected by centrifugation (1500 rpm, IEC centrifuge) in a 15-ml sterile tube (Corning Incorporated, Corning, NY). Cells were resuspended in 2.7 ml of complete RPMI 1640 containing 15 mM β-mercaptoethanol. Cell suspensions were mixed with the calcium/DNA precipitate followed by incubation for 5 min on ice, and then for 2 to 3 h at 37°C. The transfected FDC-P1 cells were washed twice with 15 ml of 1× PBS. After transfection, cells were allowed to recover for 16 h and 0.4 μg/ml puromycin was added to select for cells containing the pRetroON construct. Puromycin-resistant colonies were pooled to obtain two polyclonal cell lines containing either pRetroON/IRF-2 (F2 cells) or the parental pRetroON vector (F0 cells).

Cell Synchronization. F2 and F0 cells were synchronized by isoleucine deprivation, which arrests cells in G_1 phase. Actively growing cells (early-to-mid-log phase) were precultured for 30 h in isoleucine-deficient RPMI 1640 (Life Technologies, Inc.) with supplements. At time zero, cells were released from arrest by the addition of RPMI 1640 with supplements, with or without 25 ng/ml DOX (to induce IRF-2) for 72 h. Cells were harvested at selected time points for S1 nuclease protection assays, Western blot analysis, and FACS analysis.

S1 Nuclease Protection Assay. DNA oligonucleotides complementary to the mouse homologue of the human *FO108 H4* gene and the mRNA coding sequence of the *β-actin* gene (internal control) were used as probes to monitor RNA levels as described previously (12). In brief, hybridization was performed overnight with each of the γ-^{32}P-oligonucleotide probes (10 fmoles) and 10 μg of RNA at 16°C in hybridization buffer [80% formamide, 100 mM sodium citrate, 300 mM sodium acetate, 1 mM EDTA (pH 6.4)]. S1 nuclease digestions were performed at 37°C for 40 min using 0.4 units of S1 nuclease per sample in 200 μl of S1 nuclease buffer [262.5 mM NaCl, freshly added 0.05 mg/ml BSA, 1 mM $ZnCl_2$, 37.5 mM sodium acetate (pH4.5)]. S1 protected DNA fragments were electrophoretically separated for 2 h at 60W in a 6% denaturing polyacrylamide gel (SequaGel), and signals were quantitated using the STORM B40 Scanner.

Flow Cytometric Analysis. At the indicated time points, F2 and F0 cells were harvested, washed with PBS, and fixed with 70% ethanol at −20°C for at least 2 h. Cells were harvested by centrifugation and resuspended in a buffer consisting of nine parts 0.05 M Na_2HP0_4 and one part 25 mM citric acid containing 0.1% Triton X-100 (pH 7.8). Samples were incubated for 30 min at 25°C to extract fragmented DNA (47). Cells were then washed with PBS and resuspended in 10 mM PIPES, 0.1 N NaCl, 2 mM $MgCl_2$, and 0.1% Triton X-100, 20 μg/ml propidium iodide (Sigma Chemical Co.), and 50 μg/ml RNase A (pH 6.8), and incubated at room temperature for 30 min. The samples were analyzed for cell cycle phase distribution or polyploidy and cell death by FACStar using the Consort 30 software (Becton Dickinson, Mountain View, CA).

Western Blot Analysis. Samples of F2 and F0 cells were harvested in parallel with those used for FACS analysis and subjected to Western blot analysis using the same methods that we described in detail previously (6). Equal amounts of total cellular protein were subjected to 10 or 12% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore Corp., Bedford, MA). Blots were incubated overnight with primary antibodies using the indicated dilutions (α-IRF-2 at 1:3000, α-p53 at 1:1500, α-FAS-L at 1:1000, α-Bax and α-Bcl2 at 1:2000, α-cyclin A and α-cyclin B1 at 1:1500, α-CDK1 at 1:3000, α-CDK2 at 1:5000, α-p21 at 1:500–1000, α-p27 at 1:3500, and α-actin at 1:2500). Rabbit polyclonal antibodies (Fas-L, Bax, Bcl-2, CDK2, and p27), mouse monoclonal antibodies (p53, cyclin A, cyclin B1, and p21), and a goat polyclonal antibody (actin) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The anti-IRF-2 polyclonal antibody was described previously (5). Blots were further incubated with diluted (1:8,000–10,000) horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Inc.). Immunoreactive protein bands were visualized by enhanced chemiluminescence detection (ECL kit; Amersham Pharmacia Biotech Inc., Piscataway, NJ). Signal intensities were quantitated by densitometry, and each experiment was repeated at least three times.

RESULTS

Elevation of IRF-2 Levels Up-Regulates *Histone H4* Gene Expression at the G_1-S-Phase Transition. To examine the role of IRF-2 in regulating cell cycle progression, we prepared a stable FDC-P1 cell line containing an integrated DOX-responsive IRF-2 vector that permits forced expression of IRF-2 on DOX administration. FDC-P1 cells exhibit normal cell cycle control of histone gene transcription (43, 44); hence, the cell growth properties of FDC-P1 cells may approximate the phenotype of normal hematopoietic cells. We determined that low (*e.g.*, 1 pg/ml) and high (*e.g.*, 100 ng/ml) doses of DOX elevate IRF-2 levels to, respectively, 3- and 5-fold above normal endogenous IRF-2 levels. We also established that the IRF-2 construct mediates transcriptional activation of the *histone H4* gene promoter in a DOX-dependent manner (data not shown).

We assessed the effect of elevating IRF-2 levels on endogenous gene expression of a representative IRF-2-responsive target gene (*i.e.*, the mouse homologue of the DNA replication-dependent *histone H4/n* gene, also designated *H4.1*) that is normally induced at the G_1-S-phase transition (12). FDC-P1 cells were synchronized by isoleucine deprivation (44, 48), and aliquots of cells were harvested at multiple time points after release from the cell-cycle blockade. Samples were analyzed by Western blot analysis to measure IRF-2 levels (Fig. 1, *A* and *B*) and by S1 nuclease protection analysis to assess endogenous H4 mRNA levels (Fig. 1, *C* and *D*). Western blot analysis shows that F2 cells containing the IRF-2 expression vector have higher levels of IRF-2 than F0 cells carrying the empty parental expression vector (Fig. 1*A*, compare rows 1 and 2 with rows 3 and 4). Furthermore, F2 cells cultured in the presence of DOX (25 ng/ml) express higher levels of IRF-2 than do F0 cells cultured in parallel under the same conditions (Fig. 1*A*, compare rows 1 and row 3). However, apart from these intended DOX-dependent differences in the levels of IRF-2, we note that IRF-2 protein levels are also regulated during the cell cycle irrespective of the presence of DOX (Fig. 1*A*, *row 4*), and that the presence of DOX has a modest stimulatory effect on endogenous IRF-2 levels (Fig. 1*A*, *row 3*). Thus, there are at least three factors that contribute to the steady-state levels of IRF-2 in synchronized FDC-P1 cells: presence of IRF-2 expression vector; cell cycle stage; and presence of DOX. However, quantitation of the Western blot signals (Fig. 1*B*) reveals that IRF-2 levels are consistently higher in F2 cells cultured in the presence of DOX than F2 cells cultured without DOX, or F0 cells that lack the IRF-2 expression construct. The DOX-dependent elevation of IRF-2 levels in F2 cells results in a significant increase in expression of the *histone H4/n* gene based on S1 nuclease protection analysis, and this effect on *H4/n* gene expression is not apparent in F2 cells cultured without DOX or in F0 cells (Fig. 1, *C* and *D*). We conclude that DOX elevates IRF-2 expression in F2 cells above physiological levels, with a functional effect on the expression of a cell cycle-controlled and IRF-2-responsive target gene.

Normal Cell Cycle Progression beyond the G_1-S Phase Transition in FDC-P1 Cells Expressing Supraphysiological Levels of IRF-2. We analyzed whether elevation of IRF-2 levels during G_1 and the subsequent S phase affects cell cycle progression by evaluating the cell cycle distribution of FDC-P1 cells using FACS analysis (Fig. 2*A*). The F2 and F0 cell lines were synchronized by isoleucine deprivation and cultured in the presence or absence of DOX. The results show that the proportion of cells in S phase is very similar in F0 cells independent of

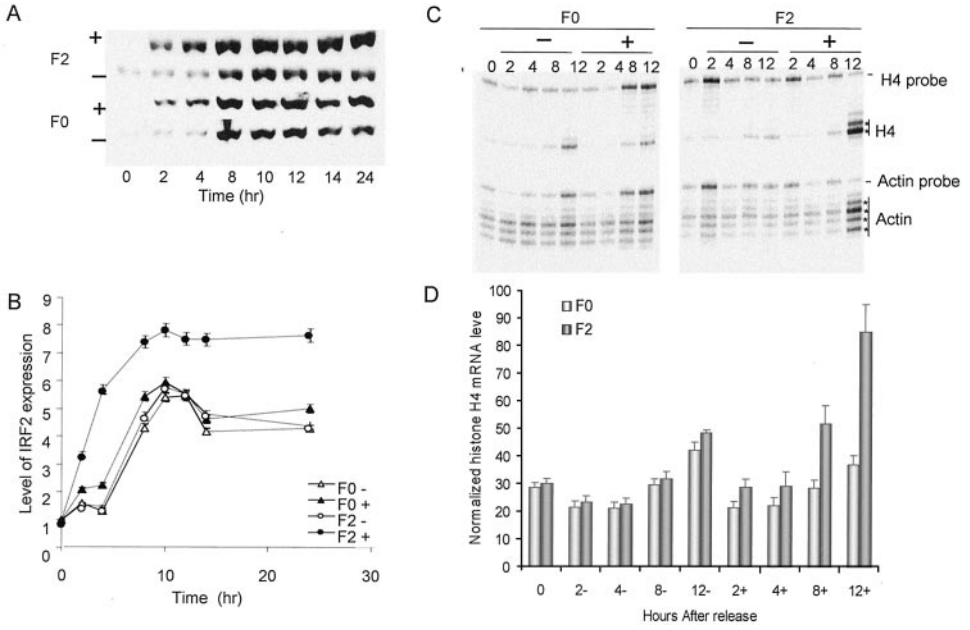

Fig. 1. Forced expression of IRF-2 up-regulates *histone H4* gene expression at the G_1-S-phase transition. *A*, Western blot analysis with an IRF-2 antibody using lysates from F2 and F0 cells at different times after release from synchronization in G_1 phase by isoleucine deprivation. IRF-2 expression was induced by DOX addition at 0 h (see Fig. 2*B*) +, induced with 25 ng/ml DOX; −, noninduced. *B*, quantitation of the Western blot data presented in *A*. *C*, S1 nuclease protection assay of RNA samples isolated at different times during cell cycle progression using radio-labeled oligonucleotides (*H4 probe* and *Actin probe*) detecting *histone H4* and actin mRNAs. ∗, the main S1 nuclease protected products for each probe, and either the mouse *H4.1* gene or the *β-actin* gene as described previously (12). *D*, graph showing the levels of *histone H4* mRNA normalized relative to *β*-actin mRNA in F0 and F2 cells in the absence (−) or presence (+) of DOX; quantitation of the S1 nuclease data from *C* was performed using a STORM B40 scanner.

the presence of DOX and in F2 cells grown in the absence of DOX at all of the time points examined between 4 and 16 h after cell cycle entry (Fig. 2*B*). The proportion of F2 cells in S phase seems to be modestly reduced in the presence of DOX (*i.e.*, ~10% decrease) relative to F2 cells grown without DOX or to F0 cells (Fig. 2*B*). We conclude that a small subset of IRF2-expressing cells may have lost the ability to enter S phase, perhaps because of a specific cell cycle block. However, the majority of F2 cells that express IRF2 at elevated levels retain the ability to progress unimpeded beyond the first G_1-S-phase transition.

Deregulated Expression of IRF-2 Causes Polyploidy and Cell Death in FDC-P1 Hematopoietic Progenitor Cells. We next assessed whether sustained elevation of IRF-2 in FDC-P1 cells influences cell growth-regulatory parameters and the cellular morphology of FDC-P1 cells (Figs. 3 and 4). The cell cycle distribution of F2 and F0 cells was analyzed by FACS in the presence or absence of DOX (Fig. 3). We validated that IRF-2 levels are only increased in F2 cells cultured in the presence of DOX and are not changed in F0 cells irrespective of the presence of DOX (Fig. 3*A*). For comparison, CDK2 levels were not altered in either F2 or F0 cells (Fig. 3*A*). The results from FACS analysis reveal that continuous up-regulation of IRF-2 above normal endogenous levels for 2–3 days causes an increase in polyploid cells (4*n* or 8*n*; Fig. 3*B*, *bottom row*, *right panel*). Increased polyploidy occurs concomitantly with a decrease in the percentage of cells in S phase and an increase of cells in G_2-M (Fig. 3*C*). Apart from polyploidy (Fig. 3*D*), we also observed a significant fraction of cells with a genome content of less than 2*n* (Fig. 3, *B*, *bottom row*, *right panel*, and *E*). Microscopic examination of F2 cells cultured in the presence of DOX reveals many cells that are larger than normal (Fig. 4, *right bottom panel*), consistent with an increase in polyploid cells and/or cells in G_2-M phase. In addition, cell death is evident from an increase in small and partially fragmented cells in the same preparation (Fig. 4, *right bottom panel*). Taken together, our results indicate that deregulation of the level of IRF-2 alters the cell growth properties of FDC-P1 cells and results in polyploidy and cell death.

Forced Expression of IRF-2 in FDC-P1 Cells Elevates FAS-L, the Activating Component of the Fas/FasL Apoptotic Pathway. To understand the mechanism by which IRF-2 causes polyploidy and cell death in FDC-P1 cells, we performed Western blot analyses using

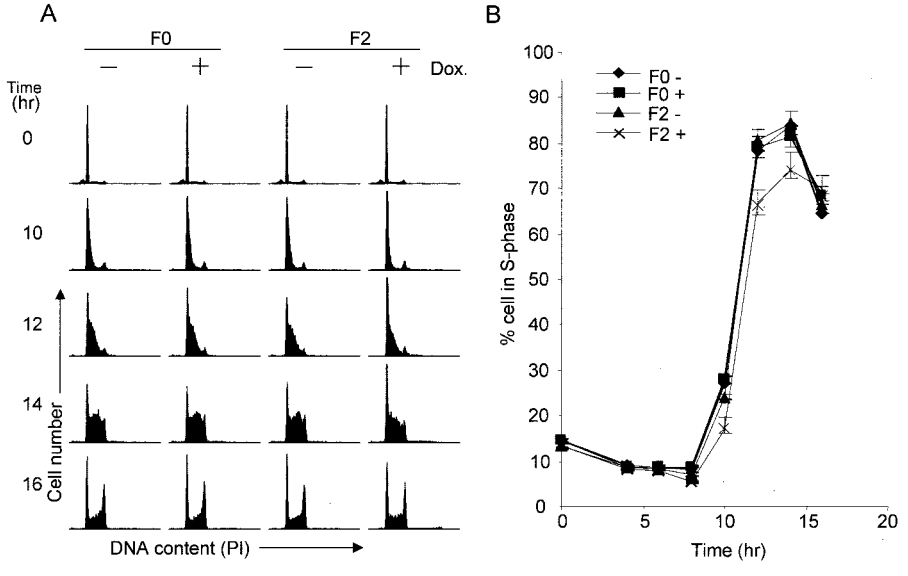

Fig. 2. Retention of competency for cell cycle progression through the first S-phase transition after forced expression of IRF-2. *A*, flow-cytometric analysis of F2 and F0 cell lines cultured in the presence (+) or absence (−) of DOX and analyzed at different times after release from isoleucine block The graphs show the number of cells (*vertical axis*) with different DNA contents (*horizontal axis*) as measured by propidium iodide (*PI*). *B*, graph showing the percentage of F2 and F0 cells in S phase (*vertical*) in the presence or absence of DOX at different times (*horizontal*) during cell cycle traverse.

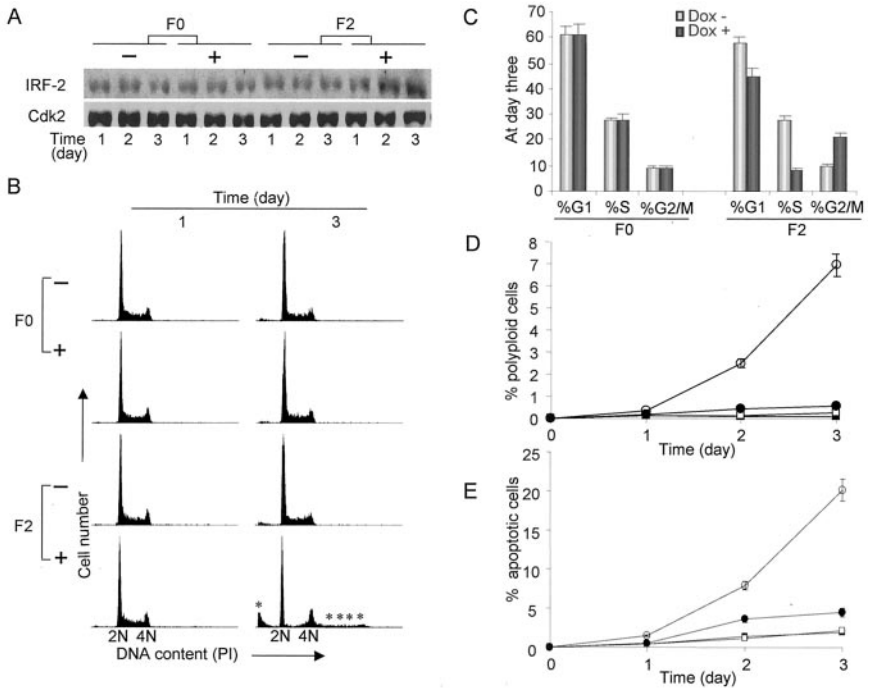

Fig. 3. Apoptosis and polyploidy of FDC-P1 cells during prolonged forced expression of IRF-2. F0 and F2 cells were synchronized and then cultured in conditioned medium (see "Materials and Methods") in the absence (−) or presence (+) of DOX for several days. *A*, Western blots showing a comparison of IRF-2 levels in F2 and F0 cells at the indicated days of treatment. CDK2 levels were monitored as internal control; forced IRF-2 expression does not cause a change in CDK2 protein levels. ***B,*** flow-cytometric assays reveal an increased proportion of cells exhibiting apoptosis or polyploidy only in F2 cells in which IRF-2 levels are elevated by DOX induction, but not in noninduced F2 cells or F0 cells under inducing or noninducing conditions. *C*, graph showing the percentage of cells in the G_1, S phase, or G_2-M at day 3 after DOX administration in F0 and F2 cells. The percentage of F0 (*squares*) and F2 (*circles*) cells exhibiting polyploidy **(***D*) or undergoing apoptosis **(***E*) at different days in culture in the presence (*open symbols*) or absence (*closed symbols*) of DOX. The graphs are based on results from at least three independent flow-cytometric assays.

antibodies against cell cycle regulatory proteins (*i.e.*, cyclins A and B1, CDK1, and CDK2, as well as the CDK inhibitors p27 and p21) and apoptosis-related factors (p53, FAS-L, Bax and Bcl-2; Fig. 5). As expected, DOX increased IRF-2 levels in F2 cells (Fig. 5*A*). We find that the levels of the cell cycle-regulatory proteins cyclin B1 and the CDK-inhibitory protein p27 are selectively increased after DOX enhancement of IRF-2 levels (Fig. 5*B*). However, in the same cells, there are no appreciable differences in the levels of CDK1 (CDC2), CDK2, and cyclin A, whereas the CDK-inhibitory protein p21 is not detected (Fig. 5*B*). The selective increase in the levels of cyclin B1 and p27 indicate that forced expression of IRF-2 arrests cells in G_2-M. These

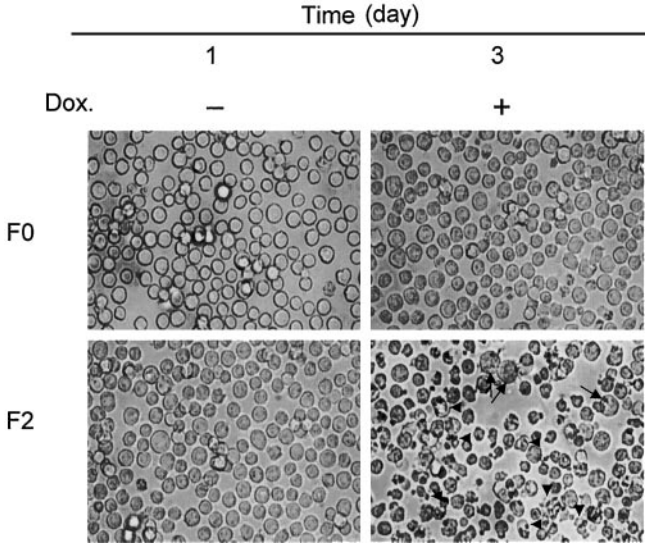

Fig. 4. IRF-2-dependent alterations in cellular morphology of FDC-P1 cells. Cell death and polyploid morphology in IRF-2-overexpressing F2 cells. The F0 and F2 cells were cultured in the absence (−) or presence (+) of 25 ng/ml DOX for 3 days. Photographs were taken using a phase-contrast microscope with a ×100 objective. Cell remnants attributable apoptosis (*arrowheads*) and polyploid cells (*arrows*) are indicated. Alterations in cellular morphology were observed only in F2 cells in which IRF-2 levels were elevated, and not in noninduced F2 cells nor in induced and noninduced F0 cells.

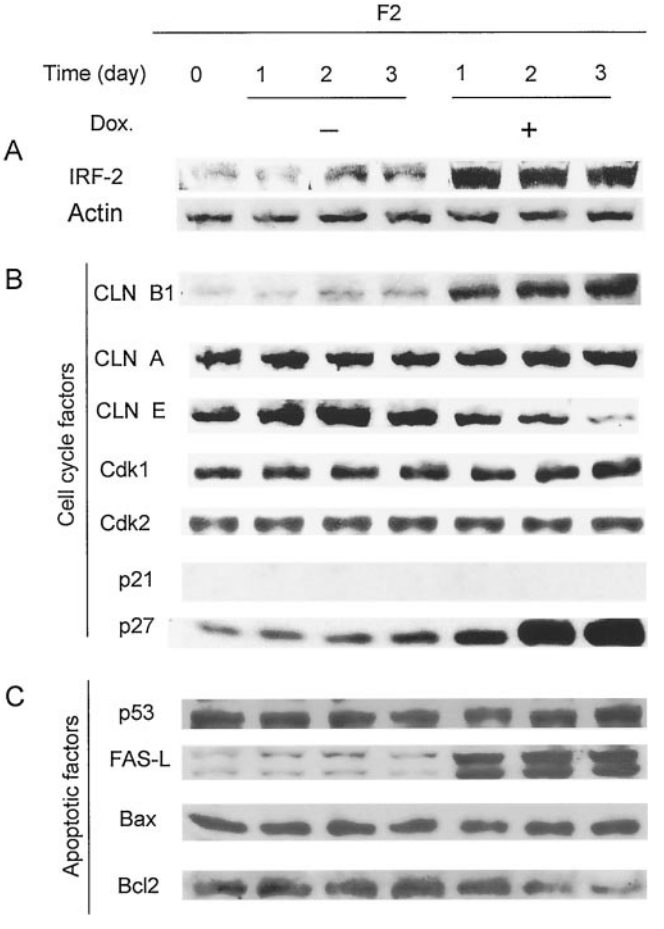

Fig. 5. Modulations in the level of cell cycle- and apoptosis-related factors by IRF-2 induction in FDC-P1 cells. F2 cells were cultured in complete RPMI 1640 plus IL-3-conditioned medium in the absence (−) or presence (+) of 25 ng/ml DOX. Whole-cell lysates, isolated at 1, 2, or 3 days after plating, were analyzed by Western blot with the indicated antibodies. No signal was observed for p21 (*empty strip*), whereas p27 levels were readily detectable; β-actin was used as control for equal protein loading.

results suggest that IRF-2 alters a mitosis-related checkpoint that may be mechanistically linked to the increase in polyploidy.

We observe that increased IRF-2 expression causes a striking elevation in the levels of the FAS-L protein that activates the FAS (Apo1/CD95) pathway (Fig. 5*C*). There is also a change in the ratio of the levels for the antiapoptotic Bcl-2 protein (modest decrease) and the apoptosis-promoting Bax protein (no major difference; Fig. 5*C*). For comparison, the levels of the tumor suppressor protein p53 remain similar in the absence or presence of DOX (Fig. 5*C*). Thus, the data of Fig. 5 and those presented in Fig. 4 indicate that IRF-2-induced cell death occurs concomitantly with elevation of a component of the Fas/FasL apoptotic pathway in FDC-P1 cells.

DISCUSSION

In this study, we characterized the IRF-2 oncoprotein as a component of an E2F-independent mechanism that controls cell proliferation. Our results indicate that deregulated expression of IRF-2 alters cell growth control of hematopoietic progenitor cells. We found that forced expression of IRF-2 does not affect progression through the G_1 and S phase stages of the cell cycle but alters cell growth by increasing the proportion of G_2-M cells, as well as by causing polyploidy and cell death. Cells that express IRF-2 above physiological levels exhibit increased expression of cyclin B1 and the CDK-inhibitory protein p27. Because cyclin B1 activates the mitotic CDK1(CDC2), and the p27 protein is capable of inhibiting CDK1 activity, these data indicate that cells become arrested in G_2-M. Forced expression of IRF-2 also increases the number of tetraploid cells, which implies a defect in chromosomal segregation. A subset of these cells exhibit a DNA content $>4n$, which indicates that cells retain competency to initiate a second round of DNA synthesis. Taken together, our findings suggest that forced expression of IRF-2 compromises a mitotic checkpoint (49), thereby creating a cell cycle defect that causes polyploidy and cell death.

Polyploidization is observed during megakaryocytic differentiation, but does not normally occur in other hematopoietic cells (50). The formation of polyploid FDCP-1 cells by forced elevation of IRF-2 levels is related to failure to undergo normal mitosis. The resulting tetraploidy may activate a p53-dependent cell cycle checkpoint in G_1 (51), and this could trigger apoptosis. However, because tetraploid IRF-2-overexpressing cells can reduplicate their genome to form polyploid ($>$4n) cells, these cells do not appear to arrest in G_1. Apart from p53 (52), several other transcription factors including c-Myc (53), p120E4F (54), and Trident (54) are involved in the formation of polyploid cells. For example, overexpression of the c-myc oncoprotein causes polyploidy in fibroblasts and apoptosis, and then proceeds in normal primary but not immortalized fibroblasts (53). Hence, c-myc and IRF-2 may share the ability to generate genomic instability when their expression is deregulated.

Hematopoietic stem cells represent uncommitted and undifferentiated blood cell precursors that respond to environmental signals by proliferative expansion and subsequent differentiation into mature cells. FDC-P1 cells are mouse myeloid progenitor cells that strictly depend on IL-3 for their survival. Absence of cytokines can trigger cell death in FDC-P1 cells, although there are FDC-P1 sublines that are at least partially resistant to apoptosis (55, 56). The IRF-2-dependent up-regulation of the CDK-inhibitory protein p27 in FDC-P1 cells, which occurs in parallel with increased apoptosis, could cause growth factor desensitization by interfering with the activities of CDKs (*e.g.*, D-type cyclins and CDK4/6) that integrate growth factor-dependent signaling pathways during G_1. However, results from our cell synchronization studies with FDC-P1 cells that are blocked in G_1 reveals that competency for cell cycle progression through G_1 and S phase occurs irrespective of elevations in IRF-2 levels. Therefore, we favor the interpretation that elevation of IRF-2 does not directly affect cytokine-dependent signaling in FDC-P1 cells.

IRF-2 may have a specific role in cell death by apoptosis. For example, IRF-2 is up-regulated during the apoptotic stage of osteoblast differentiation that occurs as a normal component of the maintenance of bone tissue organization (57). In FDC-P1 hematopoietic progenitor cells, IRF-2 may directly trigger apoptosis by regulating components of the Fas (APO-1/CD95) apoptotic pathway. Fas (APO-1/CD95) is a cell surface receptor that can mediate apoptosis and is related to the tumor necrosis factor receptor family. Cells can undergo apoptosis on expression and binding of the FasL to the Fas receptor. It has previously been shown that FasL expression in T cells is transcriptionally regulated by IRF transcription factors, and that IRF-1 and IRF-2 are each capable of interacting with the FAS-L promoter and activating FasL expression (58). Consistent with these findings, our data show that IRF-2 expression triggers a dramatic up-regulation of FasL in FDC-P1 cells, and this may activate the Fas/FasL apoptotic pathway. The product of the proto-oncogene *Bcl-2*, a membrane-associated protein, may interfere with Fas-dependent apoptosis (55, 56), and we found that overexpression of IRF-2 in FDC-P1 cells results in down-regulation of Bcl-2. Taken together, one model that can account for our findings is that the emergence of polyploidy, together with IRF-2 mediated changes in FasL levels and the concomitant activation of the FAS apoptotic pathway, may be responsible for cell death.

IRF-2 is normally up-regulated by IRF-1 in direct response to the antiproliferative actions of IFNs. Initial studies suggested that the main function of IRF-2 is to attenuate IRF-1 dependent gene activation (reviewed in Ref. 3). Our study indicates that conditional up-regulation of IRF-2 induces cell death by enhancing the endogenous levels of FasL. Hence, one important physiological function of IRF-2 is that it may extend the antiproliferative signal of IFNs by promoting conditions conducive of apoptosis. In summary, we have shown here that unbalanced expression of IRF-2 influences proliferative expansion of FDC-P1 hematopoietic progenitor cells by causing polyploidy and inducing apoptosis. Thus, our data provide important new evidence for the cell growth regulatory properties of IRF-2.

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