Requirement for Mdm2 in the Survival Effects of Bcr-Abl and Interleukin 3 in Hematopoietic Cells

Alexander W. Goetz, Heiko van der Kuip, Ruth Maya, Moshe Oren, and Walter E. Aulitzky

ABSTRACT

The p53/Mdm2 pathway plays an important role in the induction of cell cycle arrest or apoptosis in response to genotoxic stress. Both the oncogene Bcr-Abl and physiological growth factors such as interleukin (IL)-3 can modulate the outcome of cellular exposure to DNA damage. To determine whether Bcr-Abl and growth factors can affect the p53/Mdm2 pathway, we studied the expression of Mdm2 in the IL-3-dependent pre-B cell line BaF3 and its bcr-abl-transfected derivative BaF3p185 after IL-3 deprivation or treatment with the c-Abl tyrosine kinase inhibitor STI571. We found that both growth factor withdrawal and inhibition of Bcr-Abl kinase lead to a down-regulation of Mdm2 preceding the induction of apoptosis. Apoptotic cell death induced by STI571 is partially dependent on p53. The early decrease of Mdm2 protein was not attributable to transcriptional regulation or to caspase-mediated cleavage. On the other hand, it could be completely blocked by the proteasomal inhibitor lactacystin. Targeted down-regulation of Mdm2 in Bcr-Abl protein by antisense oligodeoxynucleotides overcame the survival effects of IL-3 and Bcr-Abl and resulted in accelerated apoptosis. Taken together, survival signals provided either by physiological growth factors or by oncogenic Bcr-Abl can positively regulate Mdm2, whereas Mdm2 ablation can reduce cell survival. These findings imply that, similarly to physiological growth factors such as IL-3, Bcr-Abl can promote cell survival through modulating the p53-Mdm2 pathway.

INTRODUCTION

Bcr-Abl is a chimeric protein generated by a reciprocal translocation t(9;22) in hematological disorders such as CML and a subset of acute lymphoblastic leukemia. Bcr-Abl exhibits deregulated tyrosine kinase activity and transforms both fibroblasts and hematopoietic cells in vitro and in vivo (1-4). Bcr-Abl protects cells from apoptosis (5-7) and causes cell cycle progression independent of physiological growth factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9). The mechanisms by which Bcr-Abl produces the growth stimulatory and antiapoptotic effects are factors such as IL-3 or GM-CSF (4, 8, 9).

CML is characterized by abnormal expansion of the Bcr-Abl-positive clone. Subsequent genetic damage causes transformation of the chronic disease into various acute leukemias referred to as blast crisis (16). The molecular mechanisms responsible for the genetic instability in this disease are not fully elucidated. Expression of Bcr-Abl in cultured cells leads to accumulation of genomic abnormalities, supporting the possibility of a direct contribution of Bcr-Abl to this process (17).

The p53 pathway is critically involved in the control of genomic stability in mammalian cells. Germ-line mutational inactivation of p53 predisposes to the development of hematopoietic malignancies in human Li-Fraumeni syndrome patients (18). This raises the possibility that aberrations in the p53 pathway may also contribute to the onset or progression of human leukemia.

Genetic alterations of the p53 gene are very rare in chronic phase but become more common in the blast crisis in CML (19-23). However, several lines of evidence suggest a functional interaction between p53 and Bcr-Abl. Loss of function of the p53 protein increases the transforming potential of Bcr-Abl (24). The Bcr-Abl kinase down-regulates the p53 protein in a multитarget hematopoietic cell line (25). Interestingly, the nononcogenic normal c-Abl protein can stabilize and activate p53 (26). It is also noteworthy that loss of p53-mediated apoptosis is involved in lymphomagenesis by the Abelson murine leukemia virus, which encodes v-Abl (27). In addition, p53 has been shown to play a role in the apoptotic effect of IL-3 withdrawal. IL-3 is required for the survival of mouse lymphoma DA-1 cells. In these cells, IL-3 withdrawal leads to the onset of rapid apoptosis in the presence of functional p53, whereas apoptosis is delayed when p53 is inactivated (28). On the other hand, IL-3 regulates the outcome of p53 activation in cells exposed to DNA damage. In the absence of IL-3, such cells succumb to rapid apoptosis, whereas in the presence of IL-3, they survive and undergo a viable growth arrest (29, 30). Similar observations were made also with other hematopoietic survival factors (reviewed in Ref. 31).

The Mdm2 protein is a key regulator of p53 function (reviewed in Refs. 32-34). Mdm2 binds to p53 and acts as an E3 ubiquitin ligase targeting p53 to proteasomal degradation (35). In addition, Mdm2 directly inhibits the transcriptional activity of p53 (36, 37). In response to cellular stress, both proteins undergo posttranslational modifications, leading to Mdm2 inactivation and p53 stabilization and activation (38, 39). On the other hand, elevated constitutive activity of Mdm2 is expected to prevent p53 activation and is therefore likely to promote genomic instability and oncogenesis.

Mdm2 expression can be induced by bFGF (40), as well as by other growth factors (41, 42), and by activated Ras (43). The induction of Mdm2 by basic fibroblast growth factor has been proposed to exert an antiapoptotic effect (40). We therefore asked whether Bcr-Abl and IL-3 may also regulate Mdm2 and thereby contribute to the protection of hematopoietic cells from apoptosis under physiological and pathological conditions. The expression of Mdm2 was studied in two growth factor-dependent cell lines and their bcr-abl-transfected counterparts. Withdrawal of growth factors and inhibition of Bcr-Abl kinase activity were both found to cause down-regulation of Mdm2. Importantly, this down-regulation preceded the measurable onset of apoptotic events. The rapid decrease of Mdm2 was attributable to enhanced proteasomal degradation of this protein. Our data establish a direct functional link between Bcr-Abl and Mdm2 and suggest a novel mechanism through which Bcr-Abl may abrogate the function.
of p53. This mechanism may contribute to the genetically unstable phenotype of CML cells and to their remarkable resistance to killing by chemotherapy.

MATERIALS AND METHODS

Reagents. STI571, a specific inhibitor of the c-Abl and Bcr-Abl-kinase, was kindly provided by Novartis, Inc. (Basel, Switzerland). A stock solution (10 mg/ml) was prepared by dissolving the compound in DMSO:H2O (1:1) and kept at −20°C. STI571 was used at a concentration of 1 μM. The cell permeable proteasome inhibitor lactacystin (Calbiochem, San Diego, CA) was dissolved in DMSO and used at a concentration of 10 μM. The MEF inhibitor PD98059 was obtained from Calbiochem (San Diego, CA) and used at a concentration of 25 μM. The MEK inhibitor U0126 (New England Biolabs, Beverly, MA) was used at a concentration of 10 μM. PFA (Bionol, Hamburg, Germany) was dissolved in DMSO and used at a final concentration of 20 μM.

Cells and Tissue Culture Techniques. The human cell line M07e was derived from a patient with megakaryocytic leukemia; it requires GM-CSF, IL-3, or steel factor for proliferation. M07e cells were cultured in RPMI 1640 (Seromed, Inc., Berlin, Germany) supplemented with 10% FCS and 100 ng/ml of GM-CSF (Leukomax, Sandoz, Switzerland). The cell line M07-p210 was obtained from Dr. M. Hallek, Munich, Germany (44). M07-p210 cells were generated by electroporation of M07e cells with the pG2D120 plasmid and subsequent selection for G418 (400 μg/ml; Life Technologies, Carlsbad, CA) resistance. M07-p210 cells stably express p210 Bcr-Abl and are factor independent. M07-p210 cells were grown in RPMI 1640 supplemented with 10% FCS.

The parental murine pre-B lymphocyte cell line BaF3 and the p185 Bcr-Abl-expressing derivative thereof, BaF3p185, were a kind gift from J. Duyster (Munich, Germany). BaF3 cells were grown in serum-free medium (Ultra Culture; BioWhittaker). Eight hundred 10^6 cells/sample were harvested and washed once in ice-cold PBS and then washed once in cold binding buffer (10 mM HEPES, 140 mM NaCl, and 25 mM CaCl2, pH 7.4). The cell pellet was resuspended in 100 μl of binding buffer containing 5 μl of Annexin V-FITC and 10 μl of propidium iodide (50 μg/ml stock solution in PBS). The suspension was gently vortexed and incubated at room temperature for 15 min. An additional 400 μl of binding buffer was added to the cells before analysis using a FACScan and CELLQuest software (Becton Dickinson, San Jose, CA).

Treatment of Cells with Antisense Oligonucleotides. BaF3 and BaF3p185 cells were set to a density of 5 × 10^4 per ml in serum-free Medium (Ultra Culture; BioWhittaker). Eight hundred μl of this cell suspension were mixed with 150 μl of the sense (for human cells: CCT GTA AGG TGG GAG TGA TC; for murine cells: CCT GAA GGT GGG AGT GAT C) or antisense (for human cells: GAT CAC TCC CAC CTT CAA GG; for murine cells: GAT CAC TCC CAC CTT CAG G) oligonucleotide phosphorothioates (20 μM; MWG Biotech AG, Ebersberg, Germany) in a 4-mm electroporation cuvette (Peqlab, Erlangen, Germany). One min after mixing, the cells were electroporated by means of an EasyJect-electroporator using a double pulse protocol (pulse 1: 750 V, 25 μF, 99 Ω; pulse 2: 120 V, 1500 μF, 99 Ω; OptiPulse-Option). Directly after electroporation, the cells were seeded into a 75-cm² tissue culture flask containing 25 ml of serum free UltraCulture medium supplemented with 1 ng/ml of mouse IL-3 (BaF3) or without addition of growth factors (BaF3p185).

RESULTS

Abl Protein-Tyrosine Kinase Inhibitor STI571 Causes a Rapid Mdm2-Protein Down-Regulation in Bcr-Abl-positive Cells. The expression of Mdm2 in Bcr-Abl-positive cells is not dependent on the presence of growth factors (data not shown). However, inhibition of the Bcr-Abl kinase by the tyrosine kinase inhibitor STI571 caused a remarkable down-regulation of Mdm2 protein expression in bcr-abl-transfected murine BaF3 cells (BaF3p185) and in human Bcr-Abl-expressing M07e cells (M07p210) demonstrable by confocal microscopy (Fig. 1A) as well as by Western blot analysis (Fig. 1B). Reduction of IL-3 to STI571-treated BaF3p185 cells completely restored Mdm2 expression (not shown), indicating that sustained Mdm2 expression is dependent on the presence of survival signals that can be delivered either by physiological IL-3 or by oncogenic Bcr-Abl.

Down-Regulation of Mdm2 Expression upon Survival Signal Deprivation Precedes Apoptotic Events. In contrast to Bcr-Abl-expressing M07e and BaF3 cells, the corresponding parental hematopoietic cell lines are strictly dependent on the presence of appropriate growth factors. Analogue to STI571 treatment of Bcr-Abl-positive cells, withdrawal of physiological growth factors leads to a significant down-regulation of Mdm2 protein expression in Bcr-Abl-negative cells (Fig. 1B). This down-regulation was detectable by both 2A10 and 4B2 monoclonal antibodies, the cognate epitopes of which reside within the COOH-terminal half and the extreme NH2-terminal region of Mdm2, respectively (46).

We therefore conclude that sustained Mdm2 expression is dependent on the presence of survival signals that can be delivered either by physiological IL-3 or by oncogenic Bcr-Abl.

The down-regulation of Mdm2 after survival factor withdrawal was relatively rapid. Within 4 h after IL-3 withdrawal from BaF3 cells, Mdm2 levels as detected by the 4B2 antibody were reduced to 65.3% of the amount present under optimal growth conditions, going down further to 13.6% 8 h after IL-3 withdrawal (Fig. 1C, middle panel). A comparable reduction was also seen with the 2A10 antibody (Fig. 1C, upper panel). In contrast, the measurable appearance of apoptotic features was significantly slower; 6 h after IL-3 withdrawal, only 4% of the cells were Annexin-V positive (Fig. 1C, lower panel).

In BaF3p185 cells treated with STI571, both Mdm2 down-regulation and induction of apoptosis were accelerated relative to the IL-3-deprived parental BaF3 cells (Fig. 1C, upper and middle panels). A
Fig. 1. Down-regulation of Mdm2 protein subsequent to withdrawal of survival signals precedes apoptosis. A, down-regulation of Mdm2 protein after STI571 treatment of BaF3p185 and M07-p210 shown by confocal laser scan microscopy. Cells were grown with or without STI571 for the indicated time period on chamber slides coated with polylysine. Cells were then fixed with ice-cold methanol. Mdm2 was detected with 2A10 antibody and counterstained with GAM-Texas-Red. Nuclear staining was achieved using YOPRO. B, Western blot analysis of Mdm2 in M07e, M07p210, BaF3, and BaF3p185 after factor deprivation or STI571 treatment for indicated time periods. Total extracts were electrophoretically separated. Mdm2 was detected with both 2A10 and 4B2 antibody. Equal loading was controlled by reprobing with an anti-glyceraldehyde-3-phosphate dehydrogenase antibody. C, relative Mdm2 protein expression in BaF3 and BaF3p185 after IL-3 deprivation or STI571 treatment determined by densitometric analysis of Western blot analysis of three independent experiments. For detection of Mdm2, the two antibodies 2A10 (upper panel) and 4B2 (lower panel) were used. Constitutive Mdm2 signals were set to 100% (medium control), and the relative signal intensities of the later time points were calculated. The content of apoptotic cells after IL-3 deprivation (BaF3) or STI571 treatment (BaF3p185) for the indicated time points was determined by Annexin V-FITC binding and analyzed by flow cytometry (lower panel). Values reflect the means of three independent experiments; bars, SE.
marked decrease in Mdm2 protein was detectable with both 4B2 and 2A10 1 h after exposure to STI571, when no significant apoptosis could be measured by Annexin V binding. Even 2 h after addition of the drug, only a very minor fraction (3%) of the cells have become Annexin V positive (Fig. 1C, lower panel). Hence, in both cell types the down-regulation of Mdm2 protein in response to survival signal deprivation preceded the appearance of extensive apoptosis, arguing that it was not a secondary consequence of the apoptotic process.

**STI571-induced Apoptosis Is Dependent on p53 Activity.** Fig. 1C shows that treatment of Bcr-Abl-positive BaF3p185 cells with STI571 leads to an increase of the fraction of apoptotic cells. We investigated the requirement of p53 activity herein by blocking p53 function with the specific p53 inhibitor PFT-α (47). The presence of PFT-α partially inhibits apoptosis in cells treated with STI571 by ~50% (Fig. 2). Similarly, PFT-α reduces the apoptotic fraction to a comparable extent after irradiation of cells with 50 Gy (Fig. 2). Therefore, p53 function is at least partially dependent for the observed STI571-mediated cell death in Bcr-Abl-positive BaF3p185 cells.

**Mdm2 Antisense Oligodeoxynucleotides Induce Apoptosis in IL-3-stimulated BaF3 Cells and in BaF3p185 Cells.** A critical question, in view of the findings described above, was whether down-regulation of Mdm2 is sufficient to induce apoptosis in growth factor-stimulated BaF3 cells or in Bcr-Abl-driven BaF3p185 cells. To address this issue, Mdm2 antisense ODNs were used to experimentally manipulate cellular Mdm2 levels. Two h after electroporation with antisense or sense ODNs, Mdm2 protein expression was checked by immunoblotting. In both BaF3 and BaF3p185 cells, the antisense ODNs led to a significant decrease of Mdm2 protein levels, whereas such decrease was not observed with sense ODNs (Fig. 3A). Concomitantly, increased p53 protein levels were noted in cells exposed to the antisense ODNs. This is in agreement with the fact that Mdm2 promotes the degradation of p53 (48). As expected, no increase in p53 expression was observed with sense ODNs (Fig. 3A).

Importantly, the experimental down-regulation of Mdm2 induced apoptosis; this was seen both in IL-3-stimulated BaF3 cells and in Bcr-Abl-transfected BaF3p185 cells. In both cell types, significant apoptosis was seen already 6 h after exposure to antisense ODNs with a further increase after 18 h (Fig. 3B). In contrast, the introduction of sense ODNs did not increase the apoptotic cell fraction beyond the background levels seen in cells exposed to electroporation without any ODNs (Fig. 3C). Interestingly, the observation that loss of mdm2 leads to apoptotic cell death in the presence of functional p53 has also been described for p53−/−mmd2−/− fibroblasts transfected with tsp53 (49).

Taken together, down-regulation of Mdm2 is sufficient to induce p53 up-regulation and apoptosis in BaF3 and BaF3p185 cells even under conditions where they are not deprived of their respective survival signals. Moreover, Bcr-Abl-positive BaF3 cells are as sensitive as their parental cells to induction of cell death by ablation of Mdm2 expression. Therefore, stabilization of Mdm2 levels at least partially explains how physiological growth factors or transforming oncogenes such as Bcr-Abl regulate apoptosis sensitivity.

**The Down-Regulation of Mdm2 by Survival Signal Deprivation Is Mechanistically Distinct from DNA Damage-mediated Mdm2 Modification.** Shortly after exposure to genotoxic agents, Mdm2 becomes phosphorylated in a manner dependent on the ATM kinase (39). This posttranslational modification results in decreased binding of the 2A10 antibody and appearance of a faster migrating Mdm2
form, detectable by other antibodies (39). In agreement with these observations, we could demonstrate a diminished 2A10 signal and a conspicuous alteration of the electrophoretic mobility of the Mdm2 protein detectable with the 4B2 antibody in cells exposed to ionizing radiation. These alterations could be observed already as early as 10 min after irradiation (Fig. 4, Lanes 2, 6, 8, and 12) and were seen also in cells deprived of survival signals (Fig. 4, Lanes 4 and 10). Survival signal deprivation alone, however, resulted neither in loss of 2A10 immunoreactivity nor in a change in electrophoretic mobility (Fig. 4, Lanes 3 and 9). In contrast, as a consequence of IL-3 deprivation in BaF3 or STI571 treatment in BaF3p185, there was a significant diminution of the Mdm2 signal with both antibodies, indicating an actual loss of the protein after survival signal withdrawal. Hence, the down-regulation of Mdm2 by survival signal deprivation is mechanistically distinct from the putative inactivation of Mdm2 by DNA damage such as ionizing radiation.

The Rapid Decrease of Mdm2 after Survival Signal Deprivation Is Not Attributable to Altered Transcription or Mitogen-activated Protein Kinase Activity. It has been shown previously that Mdm2 expression can be induced by constitutive activation of the Ras pathway (43). This induction involves enhanced mdm2 gene transcription and is mediated through the Raf/MEF/ERK arm of the Ras pathway (43). Because this pathway is pivotal for the signaling of both IL-3 and Bcr-Abl, we investigated whether the decrease of Mdm2 protein in BaF3 and BaF3p185 cells deprived of survival signals is also attributable to reduced transcription, and whether inhibition of MEK causes a comparable decrease of Mdm2 protein in these systems. As seen in Fig. 5A, treatment of BaF3p185 cells with STI571 led to only minor changes in mdm2 mRNA within the first 2 h, unlike the remarkable decrease of Mdm2 protein levels at the same time points. Hence, the reduction in Mdm2 protein is not attributable to reduced transcription.

To investigate a possible role of the Raf/MEK/ERK pathway in the decrease of Mdm2 protein, BaF3 and BaF3p185 cells were cultured in the presence or absence of the MEK inhibitors U0126 and PD98059. In contrast to factor deprivation (BaF3) or STI571 treatment (BaF3p185), Mdm2 protein expression did not change significantly upon addition of the MEK1/MEK2 inhibitor U0126 or the MEK1 inhibitor PD98059 for a comparable time period (Fig. 5B). The inhibitors were effective in the BaF3 and BaF3p185 cells, because both inhibitors elicited a significant reduction in phosphorylated ERK1/ERK2 (Fig. 5B). We therefore conclude that, in BaF3 and BaF3p185 cells, modulation of the Ras/MEK/ERK pathway is not responsible for the decrease of Mdm2 protein in the absence of survival signals.

Survival Factor Withdrawal Leads to Rapid Proteasomal Degradation of Mdm2. To gain insight into the mechanism responsible for the down-regulation of Mdm2, the levels of this protein were analyzed after survival signal deprivation in the presence of lactacystin, a potent inhibitor of the 26S proteasome. Lactacystin completely blocked the rapid down-regulation of Mdm2 by STI571 in BaF3p185 cells (Fig. 6). Similarly, the decrease of Mdm2 in parental BaF3 cells after withdrawal of IL-3 was also inhibited by lactacystin (not shown). Treatment of cells with lactacystin alone led to a similar Mdm2 expression, as shown in Fig. 6.
We conclude that both Bcr-Abl and IL-3 maintain high Mdm2 levels by stabilizing Mdm2 against proteasomal degradation.

**DISCUSSION**

Mdm2 is a key regulator of p53 (reviewed in Refs. 32–34). It is conceivable that the stoichiometric balance between the two proteins may determine the extent of cellular p53 activity and the likelihood that cells will carry out an effective p53 response when confronted with appropriate signals. Therefore, under conditions where p53 activation can lead to apoptosis, regulation of Mdm2 protein expression may constitute a major molecular switch controlling the likelihood of apoptosis.

We report here that, in two different types of ARF-negative hematopoietic cells, signals induced by Bcr-Abl and IL-3 can maintain high Mdm2 levels. Withdrawal of growth factors or inhibition of the Bcr-Abl kinase is followed by a rapid decrease of the Mdm2 protein content preceding the induction of apoptosis. Importantly, induction of a comparable decrease of Mdm2 protein by antisense ODNs is sufficient to up-regulate p53 and to initiate apoptosis in these cells, even in the presence of continuous exposure to survival signals. In addition, our results clearly indicate that the apoptotic response to STI571 in Bcr-Abl-positive BaF3 cells requires p53 function. Taken together, the mdm2/p53 system plays an important role in cell survival mediated by physiological and transforming growth signals.

The Ras/MEK/ERK pathway is one of the critical pathways implicated in the signaling of both Bcr-Abl and activated IL-3 receptors (reviewed in Refs. 50–53). It has been shown recently that exposure to the MEK inhibitor U0126 for 48 h leads to a decrease of Mdm2 content in a human colon cancer cell line (43). This down-regulation was attributable to decreased transcription of the mdm2 gene. Interestingly, our data demonstrate that the rapid down-regulation of Mdm2 within the first 2 h after deprivation of survival signals does not result from decreased transcription. Moreover, two different MEK inhibitors did not alter Mdm2 protein content, despite a very efficient inhibition of ERK phosphorylation, implying that the regulation of Mdm2 by IL-3 or Bcr-Abl within the small time frame we investigated is not dependent on the MEK pathway. We have not been able to demonstrate formally a decrease in Mdm2 protein after growth factor withdrawal or STI571 treatment. This may be attributable to the fact that the half-life of Mdm2 in untreated BaF3 cells is <10 min (data not shown), making it technically difficult to monitor a further shortening of the half life. At present, we cannot formally exclude the possibility that growth factor withdrawal or STI571 treatment decreased the Mdm2 protein levels by other mechanisms, e.g., altered translation rates. However, the luteinising effects strongly argue that proapoptosome-mediated degradation plays a major role in this effect.

Bcr-Abl does not confer resistance to cell death induced by down-regulation of Mdm2. In contrast, Bcr-Abl-positive cells have proven to be resistant to most other proapoptotic stimuli, including ionizing radiation, cytotoxic agents, or growth factor deprivation (reviewed in Refs. 54–56). Several pathways have been implicated in the antiapoptotic effects of Bcr-Abl. For instance, Bcr-Abl can activate phosphatidylinositol 3-kinase, leading to increased activity of the PKB/Akt serine threonine kinase (13). In addition, Bcr-Abl can up-regulate Bcl-XL by thus far unknown molecular mechanisms (10, 11). The fact that antisense down-regulation of Mdm2 induces apoptosis, despite the presence of Bcr-Abl, suggests that Mdm2 and probably also p53 may act downstream of Bcr-Abl.

Our studies indicate that Bcr-Abl and IL-3 mediate survival in murine and human hematopoietic cells at least partly by up-regulation of Mdm2 protein expression. Presumably, survival in the presence of Bcr-Abl or IL-3 in these cells is achieved by incapacitating the p53 pathway, which plays an important role in maintaining genomic stability. Continuous stimulation of Mdm2 expression, as observed in cells with a constitutively active Bcr-Abl kinase, may thus compromise the genomic stability of these cells and thereby contribute to the accumulation of progressive DNA damage in Bcr-Abl-positive malignant disease.

Several studies suggest a major role for Mdm2 in the pathogenesis of human leukemias. Frequent overexpression of mdm2 mRNA has been described in acute and chronic leukemias (57–61). Unlike what has been observed in a variety of human solid tumors, overexpression of Mdm2 in human leukemias is typically not attributable to amplification of the mdm2 gene (58, 59, 62). Rather, high Mdm2 expression in hematopoietic malignancies is caused by enhanced transcription, enhanced translation, and prolonged protein half-life (48, 63, 64). Acute myelogenous leukemias overexpressing Mdm2 have a worse prognosis than Mdm2-negative leukemias (65). Moreover, in nonhematopoietic cell lines, down-regulation of Mdm2 has been shown to confer enhanced sensitivity to irradiation and cytotoxic agents (66, 67). Therefore, Mdm2 represents an attractive target for therapeutic manipulation in human leukemias.

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