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Research Article

Unscheduled CDK1 activity in G1 phase of the cell cycle triggers apoptosis in X-irradiated lymphocytic leukemia cells

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Abstract. Cyclin-dependent kinase 1 (CDK1) is a major component of the cell cycle progression engine. Recently, several investigations provided evidence demonstrating that unscheduled CDK1 activation may also be involved in apoptosis in cancerous cells. In this article, we demonstrate that X-ray irradiation induced G1 arrest in MOLT-4 lymphocytic leukemia cells, the arrest being accompanied by reduction in the activity of CDK2, but increased CDK1 activity and cell apoptosis in the G1 phase. Interestingly, this increase in CDK1 and apoptosis by ionizing radiation was prevented by pretreatment with the CDK1 inhibitor, roscovitine, suggesting that CDK1 kinase activity is required for radiation-induced apoptotic cell death in this model system. Furthermore, cyclin B1 and CDK1 were detected co-localizing and associating in G1 phase MOLT-4 cells, with the cellular lysates from these cells revealing a genotoxic stress-induced increase in CDK1 phosphorylation (Thr-161) and dephosphorylation (Tyr-15), as analyzed by postsorting immunoprecipitation and immunoblotting. Finally, X-irradiation was found to increase Bcl-2 phosphorylation in G1 phase cells. Taken together, these novel findings suggest that CDK1 is activated by unscheduled accumulation of cyclin B1 in G1 phase cells exposed to X-ray, and that CDK1 activation, at the wrong time and in the wrong phase, may directly or indirectly trigger a Bcl-2-dependent signaling pathway leading to apoptotic cell death in MOLT-4 cells.

Keywords. Apoptosis, G1 arrest, cyclin B1, CDK1, CDK2, lymphocytic leukemia cells, MOLT-4 cells, X irradiation.

Introduction

Apoptosis has been described as an abortive cell cycle progression event due to the morphological similarities between apoptosis and mitosis [1, 2]. A number of investigations revealed that it is the close association of genes such as Myc [3] and E2F-1 [4] that clearly link their roles in cell proliferation to apoptosis. Several studies showed that Hpo, Sav and Wts define a tumor suppression pathway that coordinately regulates cell proliferation and apoptosis [5]. Therefore, in response to genotoxic stress, it is possible that the cellular decision of life *versus* death involves an intricate network of multiple genes that play critical roles in regulation of DNA repair, cell cycle, and cell death. The challenge now faced is to understand the

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mechanisms through which these genes coordinately regulate cell fate; cellular commitment toward growth *versus* death. Although cyclin-dependent protein kinases (CDKs) play a critical role in the regulation of cell cycle progression, recent evidence suggests that they are also involved in regulation of apoptosis. For example, their unscheduled expression and inappropriate activity during cell cycle transitions often correlates with apoptotic cell death. Thus, it appears that CDKs may play a central role in the regulation and coordination of cellular proliferation and apoptosis.

However, the role of CDKs as regulators of apoptosis remains controversial. Choi and colleagues [6] showed that transient increases in the activities of CDKs were associated with a dramatic increase in TGF-β-induced apoptosis, and that CDC2 (CDK1) and CDK2 inhibitors prevented apoptosis; this was supported by similar conclusions drawn from other experimental systems [7, 8]. In contrast, it was recently reported that SU9516, a novel selective CDK2 inhibitor, induced apoptosis in colon carcinoma cells [9]. Furthermore, De Luca and coworkers [10] showed that Fas-induced changes in CDC2 (CDK1) and CDK2 kinase activities were not sufficient for triggering apoptosis in HUT-78 cells, and that these alterations in CDC2 (CDK1) and CDK2 kinase activities were only the secondary events in cell death.

All in all, many questions remain unanswered. For instance, which CDK is involved in regulation of apoptosis, and how does CDK exert its double roles as a regulator of cell cycle progression and apoptotic cell death? In an attempt to address these questions, we designed this study to assess the role of CDK1 in the regulation of apoptosis. Importantly, we found that CDK1 activity was required for X-ray radiation-induced apoptosis of MOLT-4 lymphocytic leukemia cells. Our results suggest that the unscheduled type of cyclin B1 in G1 phase causes CDK1 activation at the wrong time and in the wrong place, with the latter triggering a presently unknown signaling pathway leading to Bcl-2 phosphorylation and apoptotic cell death in MOLT-4 cells.

Materials and methods

Cells and treatment. The acute lymphocytic leukemia cell line MOLT-4 (ATCC, Manassas, VA, USA) was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. All media, supplements, and sera were obtained from GIBCO (Grand Island, NY, USA). The experiments were performed on cells during their exponential phase of growth. Cells were irradiated with 20-Gy X-rays and harvested at 3, 6, and 9 h, respectively, following the X-ray exposure, or as indicated in each experiment.

Flow cytometric analysis. Harvested cells were fixed in ice-cold 80% ethanol, at –20 °C, for at least 24 h. Sub-G1 assay for fixed cells and Annexin V assay for fresh cells were performed as described [11]. Propidium iodide (PI) staining was used to reveal DNA content and to separate the subpopulation of cells based on cell cycle phase. Immunofluorescence detection for cyclins/DNA was done as previously described [12]. Following overnight fixation, cells were washed in PBS, and permeabilized with 0.5% Triton X-100 in PBS for 5 min on ice. After centrifugation, the cells were incubated overnight in the presence of a primary antibody against cyclins (BD Pharmingen) diluted in PBS containing 1% BSA. Next, cells were rinsed, and incubated with the secondary FITC-conjugated antibody (DAKO, diluted in PBS containing 1% BSA) for 30 min. Finally, cells were resuspended in PI solution (50 µg/ml PI), and incubated at room temperature for 30 min. Cellular fluorescence was measured using a FACSVantage flow cytometer (Becton Dickinson).

Annexin V-PI assay. A new method for detection of cell cycle-specific apoptosis, Annexin V-PI (API) assay, was recently established in our laboratory [11]. In brief, 5 µl FITC-Annexin V were added to 100 µl of freshly collected cells suspended in binding buffer, at a density of 10^6 cells/ml. The cells were placed at room temperature in the dark for 20 min, rinsed in binding buffer and resuspended in binding buffer containing 1 ml of 1% methanol-free formaldehyde for 30 min on ice. Afterwards, cells were rinsed twice and resuspended in 0.5 ml PI solution containing 50 µg/ml PI, 0.1% RNase A (Sigma), 500 µg/ml digitonin (Sigma), 10 mM PIPES (Sigma), 2 mM $CaCl_2$, and 0.1 M NaCl. Resuspended cells were then stored in the dark at room temperature for 1 h prior to analysis by flow cytometry for the cell cycle specificity of apoptotic cells. Untreated cells were used as the negative controls.

Postsorting immunoprecipitation and Western blot analysis. Cells harvested at each time point were fixed in ice-cold 80% ethanol, at –20 °C, and stained with PI solution in the dark for 30 min, as described previously [13]. Cellular DNA content was quantified by the fluorescence of PI-stained DNA with the FACSVantage. Intact cells were separated to three sub-groups (G1/G0, S, and G2/M) based on DNA content, whereupon each sub-group was sorted by a FACSVantage. Cells were collected from each subgroup, adjusted to identical final cell concentrations, and lysed in lysis buffer (50 mM Tris-HCl, pH 7.4, 250 mM NaCl, 1 mM EDTA, 50 mM NaF, 1 mM DTT, 0.1% Triton X-100, 10 µM leupeptin, 100 µg/ml aprotinin, 0.5 mM PMSF) (ultrasonic 20 s at 20 W) for 20 min at 4 °C. Cell lysates were then cleared by centrifugation at 12 000 rpm and protein concentrations were quantitated. Immunoprecipitations were performed by incubating lysates with a specific antibody (2 µg/reaction) at 4 °C for

4 h, after which, 50 µl protein A–Sepharose was added to the mixture, which was further incubated for 12 h. Immune complexes were centrifuged at 5000 rpm for 5 min, and precipitates were washed three times with lysis buffer. The pellet was resuspended in 2 × SDS sample buffer, boiled for 5 min, and centrifuged at 12 000 rpm for an additional 5 min. The supernatant was collected and subjected to 12% SDS-PAGE. Proteins were transferred to PVDF membranes. The presence of co-immunoprecipitated protein was confirmed by Western blotting using specific antibodies. Detection was performed by ECL system (Amersham Pharmacia).

Immunoprecipitation and *in vitro* kinase assays. CDK1 and CDK2 activities were measured by immunocomplex assays as described previously [10]. Briefly, whole cell lysates were prepared in lysis buffer, and immunoprecipitated with various antibodies (anti-CDK1 or anti-CDK2; Santa Cruz Biotechnology). Immune complexes were recovered with the aid of protein A–Sepharose beads. The beads were washed three times with lysis buffer and twice with kinase reaction buffer (50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM EGTA, 2 mM DTT, 0.1 mM sodium vanadate, 40 mM β-glycerophosphate, 40 mM *p*-nitrophenylphosphate). Kinase reactions were performed by resuspending the beads for 30 min, at 30 °C, in 25 µl kinase reaction buffer in the presence of 0.4 mg/ml histone H1, 50 µM ATP, and 5 µCi [γ-^{32}P]ATP. The reaction was stopped by boiling the samples in 2 × SDS sample buffer followed by subjecting the samples to SDS-PAGE. Histone H1 phosphorylation was assayed by autoradiography on the gel dried after electrophoresis.

Results and discussion

The induction of cell cycle phase specific apoptosis by a variety of agents was originally demonstrated in an earlier study [4]. In addition, this phenomenon of cells to undergo apoptosis at specific phases of the cell cycle was later observed to occur in a variety of cell types [4, 6, 8]. This characteristic feature of apoptosis is considered to be the outcome of cell cycle checkpoint control. It is known that several checkpoints exist at different phases, and that each checkpoint may trigger apoptosis at a particular time point of the cell cycle. Moreover, different subtypes of CDKs may initiate cell cycle-specific apoptosis modulated via different checkpoints. However, little is known about the underlying mechanism that links cell cycle checkpoints to apoptotic cell death. Thus, the induction of cell cycle phase-specific apoptosis by genotoxic stresses through modulating checkpoints (*i.e.* regulating crossroads between cell proliferation and cell death), provides an experimental model for studying the association between cell cycle and apoptosis.

X-ray irradiation is known to induce cell death as well as cell cycle arrest through DNA damage. To study the relationship between a cell cycle checkpoint and apoptosis by X-ray radiation, flow cytometric analysis was performed. In this study, when MOLT-4 lymphocytic leukemia cells were exposed to 20 Gy of X rays and incubated for 3, 6, and 9 h, the flow cytometric profiles of this cell line showed marked increases in the G1 phase fraction, as indicated by cellular DNA content and the levels of cyclin E expression (Fig. 1a, b). This suggests the occurrence of radiation-induced G1 arrest in these cells because the detection of cyclin E level provides a sensitive marker for G1-S transition arrest [14]. Apoptosis analysis by routine Annexin V assay showed that the rate of cell death was increased rapidly at 4–6 h following a slow rise at early time of X-ray exposure (data not shown). To assess the cell cycle phase specification of apoptosis, API assay, our new method for measuring the cell cycle phase specificity of apoptosis in asynchronously growing cells, was utilized [11]. Our data showed that apoptotic cells, which were Annexin V positive, were predominantly located at the G1 position (Fig. 1c). Thus, these results indicate that X-ray treatment of MOLT-4 cells caused both G1 phase arrest and G1 phase apoptosis.

The activity of CDKs is known to be cell cycle phase specific. For example, the activity of CDK2 peaks in late G1 phase and promotes the G1-S transition, while the activity of CDK1 is maximal in G2-M phase. Since the activity of CDKs is cell cycle phase specific and most genotoxic stress-induced apoptosis is also a cell cycle phase-specific event, the strategy of investigations should be focused on a particular phase of the cell cycle to study the inner link between these events. Previously, cell cycle synchronization at the G1-S interphase by inhibitors of DNA polymerase has been used to obtain cells at a specific phase of the cell cycle. However, the procedure of synchronization itself was found to cause growth imbalance and altered expression levels of cyclins, thereby perturbing cell cycle progression [15, 16]. Recently, we developed a new method called postsorting Western blotting, which combines flow-sorting with protein analysis techniques [13]. This method can be used to detect the expression of proteins in specific cell cycle phases without cell cycle synchronization, and was therefore employed in the present study to uncover internal relations between the expression of CDKs and cell cycle phase-specific apoptosis.

CDK2 is a major CDK in G1 phase, and the activity of CDK2 plays a key role in the regulation of the G1-S transition [17]. In view of X-ray-induced G1 arrest and upregulation of cyclin E, we sought to investigate the effect of X-irradiation on CDK2 activity in MOLT-4 cells. As shown in Fig. 2a, our kinase assays demonstrated dramatically reduced CDK2 activity in X-irradiated MOLT-4 cells at 2 h that persisted at such low levels for up to 8 h. This

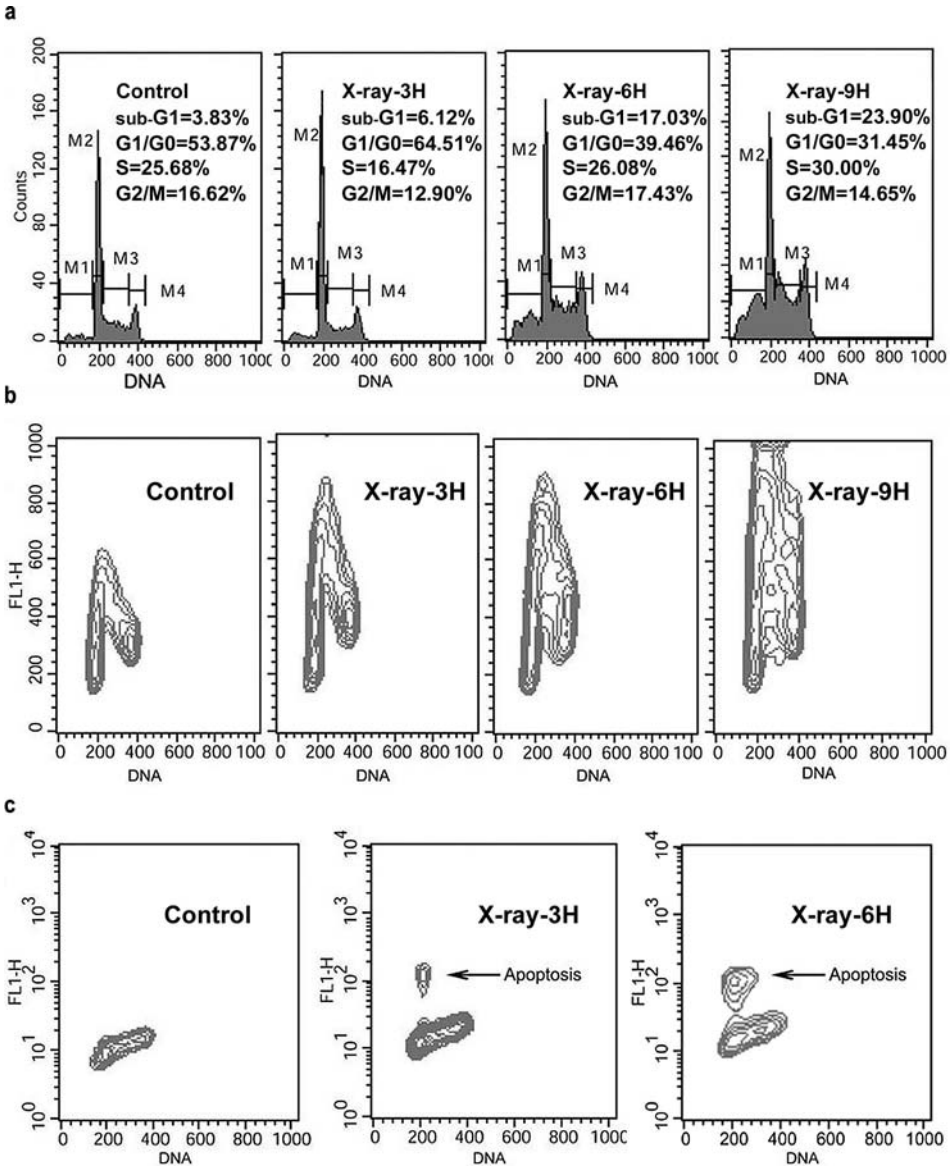

Figure 1. X-ray exposure-induced G1 arrest and G1 apoptosis in human MOLT-4 lymphocytic leukemia cells. MOLT-4 cells were exposed to X-ray irradiation (20 Gy), after which cells were harvested at different time points, as indicated. (*a*) DNA analysis by flow cytometry shows that the proportion of G1/G0 subgroup cells is increased before the appearance of the sub-G1 peak (apoptosis), while the population of G1/G0 cells is decreased when the sub-G1 peak rises. (*b*) The levels of cyclin E in the G1 phase are elevated markedly at early time points following X-ray irradiation (3 h) and remains at higher levels until apoptosis is increased later. (*c*) API assay reveals that Annexin V-positive cells (apoptotic cells) are predominantly located at the G1 position.

finding suggests that X-ray-induced cell cycle G1 arrest in MOLT-4 cells (Fig. 1) is mediated through inhibiting CDK2 kinase activity and blocking the G1-S transition. Caffeine is capable of abrogating cell cycle checkpoints in several different mammalian cell types [18, 19] by preventing CDK2 activation through the Chk1-CDC25A-CDK2 pathway [20]. In this model system, we found that caffeine (50 mM) also remarkably decreased the cyclin E threshold level and abrogated G1 arrest in response to ionizing radiation [21]. Interestingly, we also observed that MOLT-4 cell apoptosis was prevented by caffeine (data not shown). These observations thus indicate that the G1 arrest induced by X-rays is associated with G1 apoptosis, and that the cell cycle G1 arrest occurred prior to apoptosis in this system, suggesting that G1 arrest is a prerequisite for G1 apoptosis.

The role of CDK1 in the regulation of apoptosis is receiving increasing attention [1, 2]. To investigate the internal relations between cell cycle control and apoptotic cell death, we assessed the role of CDK1 activity in X-irradiation-induced apoptosis in MOLT-4 cells. *In vitro* kinase assay was performed and the data showed that X-ray-induced increases in CDK1 activity at 4 h following X-ray exposure, which positively correlated with

increases in apoptotic cell death in these cells, suggesting an important role of CDK1 in X-irradiation-induced apoptosis in this model (Fig. 2a, b). This viewpoint is supported by our demonstration that roscovitine, a potent CDK1 inhibitor, effectively blocked X-ray-induced CDK1 activity and X-ray-induced apoptosis in MOLT-4 cells (Fig. 2b). In addition, our subsequent experiments using postsorting Western blotting revealed that the increased CDK1 activity associated with the G1 phase apoptosis was detected in X-irradiated G1 phase MOLT-4 cells (Fig. 2c). These results suggest that, in addition to the normal scheduled CDK1 activity in G2-M phase as M phase-promoting factor for the G2-M transition [22], the unscheduled activity of this protein kinase may play an important role in the G1 phase specific apoptosis observed in MOLT-4 cells following exposure to X irradiation. Not surprisingly, we found that the up-regulation of cyclin B1 expression was also detected in X-irradiated G1 phase cells as compared with the untreated G1 cells (Fig. 3a). Furthermore, our postsorting co-immunoprecipitation analysis suggested that this unscheduled type of cyclin B1 was the partner and activator for CDK1 activation in the G1 phase cells (Fig. 3b). This unscheduled cyclin B1 up-regulation in G1 was abolished by caffeine, which suggests that the up-regulation of cyclin B1 in G1 is closely linked to or results from cell cycle arrest in G1 phase. Finally, the changes in the levels of phosphorylation on CDK1, at Thr-161 and Tyr-15, indicate that CDK-activating kinase, Wee1, Myt1, and CDC25 may participate in the sequential phosphorylation and dephosphorylation of CDK1 in the process (Fig. 2c). These data obtained from MOLT-4 cells were reproducible in human peripheral blood lymphocytes in our laboratory (data not shown).

In response to genotoxic stress, cells may either undergo cell cycle arrest and DNA repair or commit suicide if the damage is beyond repair. Among the many factors that influence cellular decision-making under genotoxic stress, CDKs and cyclins may play crucial roles in the regulation of cell cycle arrest and cell death. Cyclin B1 is known to form a complex with CDK1, which phosphorylates their substrates to urge cells through the G2-M transition [23]. It is believed that cyclin B1 accumulates in the S phase of the cell cycle and reaches the maximal level at mitosis but is absent in G1 phase cells. Interestingly, we recently established a new method called postsorting Western blotting for examining protein expression in specific phases of sorted, asynchronously growing cells. We showed, using this new method, that the strictly conservative protein cyclin B1 in the G2-M phase was expressed in the unscheduled mode in the G1 phase of the cell cycle in MOLT-4 cells and other solid tumor cells [13]. Although the role and mechanism of cyclin B1-CDK1 function in the cell cycle control are well known, their role and mechanism in apoptosis have yet to be completely understood.

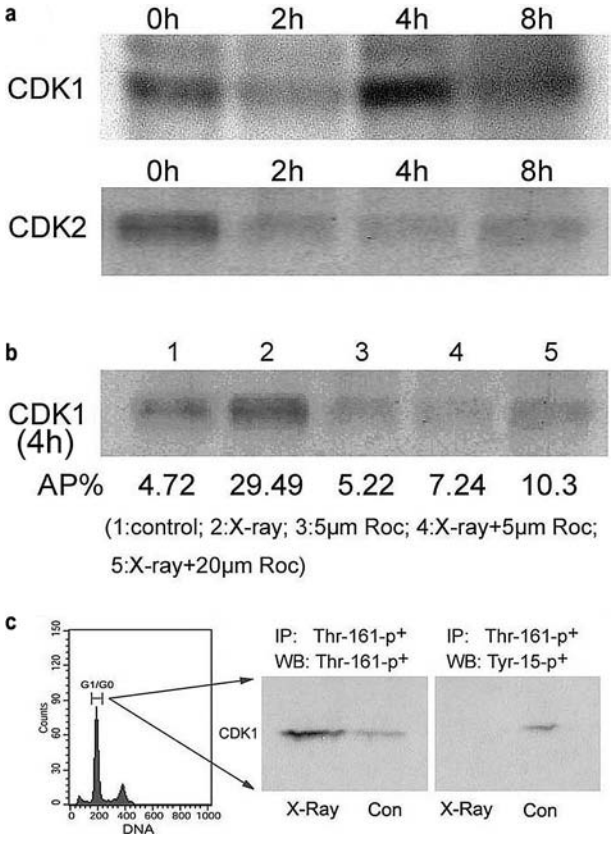

Figure 2. CDK1 and CDK2 activity in the G1 phase of human MOLT-4 lymphocytic leukemia cells after exposure to X-irradiation. (*a*) MOLT-4 cells were exposed to X-irradiation (20 Gy), and cells were then harvested at different time points, as indicated. Kinase assays show that both CDK1 and CDK2 activities are decreased at 2 h after X-ray exposure. The activity of CDK1 is increased remarkably at 4 h, while the activity of CDK2 remains at lower levels for up to 8 h. (*b*) Roscovitine (Roc) effectively blocks X-ray-induced apoptosis and CDK1 activity in MOLT-4 cells at 4 h after exposure to irradiation. The percentage of apoptotic cells (AP%) is given for different treatments of MOLT-4 cells. (*c*) G1/G0 MOLT-4 cells were collected by flow sorting and cellular lysates were immunoprecipitated with antibody against phosphorylated CDK1 (Thr-161). Western blotting was then performed using antibody against phospho-CDK1 (Thr-161) or phospho-CDK1 (Tyr-15) to detect CDK1 activity in G1/G0 cells following irradiation. Note that phospho-CDK1 (Thr-161-p+ and Tyr-15-p–) represents active CDK1, whereas phospho-CDK1 (Tyr-15-p+) is inactive.

A number of recent studies have demonstrated that CDK1-cyclin B1 also plays a critical role in regulation of apoptosis in different experimental systems [24]. Increased CDK1 activity has been observed in various apoptotic conditions [25–27]. Moreover, overexpression of active CDK1-cyclin B1 is known to promote mitotic cell death [28, 29] and inhibition of the CDK1-cyclin B1 complex by dominant negative *cdk1* mutants, antisense constructs, or chemical inhibitors prevents apoptosis [26, 30, 31]. Yet the role and importance of the unscheduled type of cyclin B1-CDK1 activity in apoptosis remain unclear. In this study, we observed X-ray-induced apoptosis in MOLT-4

cells that was associated with increased CDK1 activity and accumulation of CDK1-cyclin B1 complex in G1 phase cells, as indicated by the fact that (i) unscheduled expression of cyclin B1 was increased in G1 phase MOLT-4 cells; (ii) cyclin B1 and CDK1 were detected co-localizing and associating in the G1 phase cells following X-ray exposure, and (iii) the cellular lysates from these cells revealed increased CDK1 phosphorylation (Thr-161) and dephosphorylation (Tyr-15), as assessed by postsorting immunoprecipitation and immunoblot analysis (Figs 2 and 3). Furthermore, the CDK1-specific inhibitor, roscovitine, was able to block X-ray-induced CDK1 kinase activity and apoptosis. Additionally, our flow cytometry analysis revealed that X-ray-induced G1 apoptotic cell death occurred after the G1 arrest induced by the genotoxic agent. Therefore, we postulate that CDK1 may play a critical role in initiation of cell death by triggering, directly or indirectly, a signaling pathway leading to Bcl-2 phosphorylation/inactivation (Fig. 4) and activation of caspase and apoptosis in the lymphocytic leukemia cells. If so, fundamental mechanistic questions remain to be answered. What is the link between CDK1 and the core apoptotic machinery? How does the G1-S checkpoint system breakdown after lengthened G1 arrest to result in the unscheduled type of cyclin B1 up-regulation and CDK1 activation in G1 MOLT-4 cells? Further studies are needed to provide more definitive answers to these important questions.

The phosphatidylinositol 3′-kinase (PI3K)/AKT/PTEN pathway has been reported to be implicated in the progression of cell proliferation and the suppression of apoptosis in a variety of human malignancies and normal cells. PI3K activity has been implicated in regulating cell cycle progression at distinct points in the cell cycle by preventing cell cycle arrest or apoptosis. AKT, also called protein kinase B (PKB), is the downstream target of PI3K that plays critical roles in controlling cell proliferation

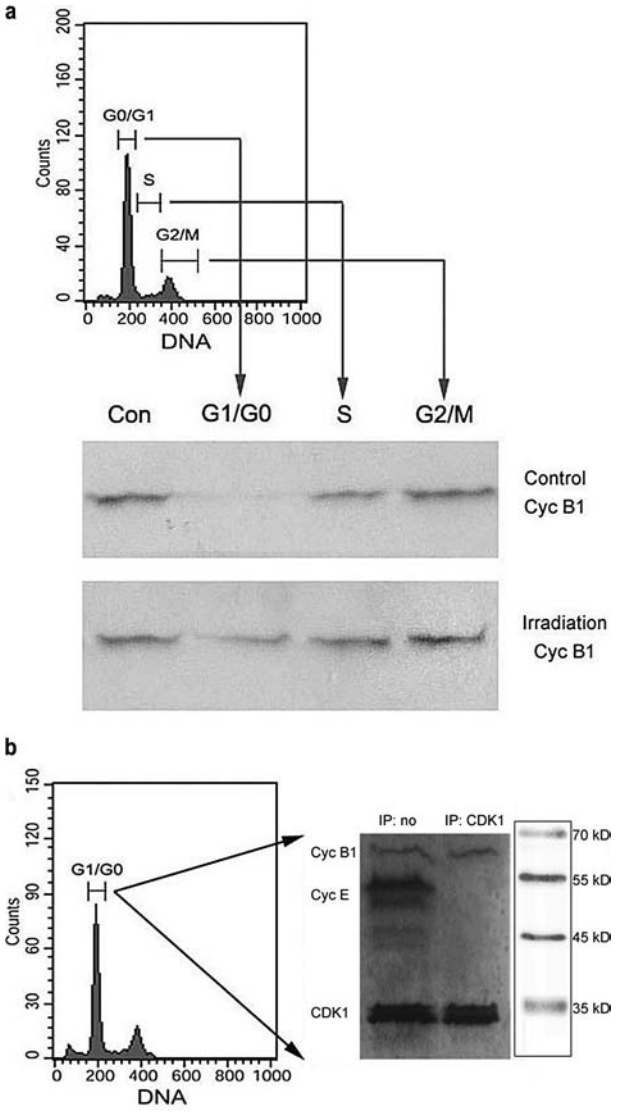

Figure 3. Detection of unscheduled type cyclin B1 expression in G1 phase of MOLT-4 human lymphocytic leukemia cells exposed to X-irradiation. (*a*) MOLT-4 cells were exposed to X-irradiation (20 Gy) for 4 h, and non-irradiated cells were used as the controls. Irradiated cells (Irradiation) were divided into three groups (G1/G0, S, and G2/M) by flow sorting. Western blot was performed to detect cyclin B1 levels in the sorted cells. Intact, unsorted cells were used as controls (Con). Our results show that scheduled expression of cyclin B1 is detected in the S and G2/M phases but not in the G1/G0 phase of non-irradiated cells (Control). In contrast, unscheduled expression of cyclin B1 is detected in G1/G0 phase MOLT-4 cells following X-ray exposure (Irradiation). (*b*) G1 phase cells were collected from irradiated cells by flow sorting. Western blot was then performed with the cell lysates of the G1 phase cells immunoprecipitated with the antibody anti-CDK1 (right lane) or without immunoprecipitation (left lane). CDK1, cyclin B1, and cyclin E were detected in G1 phase cells by Western blot analysis (left lane), while cyclin B1 was co-immunoprecipitated with CDK1 (right lane).

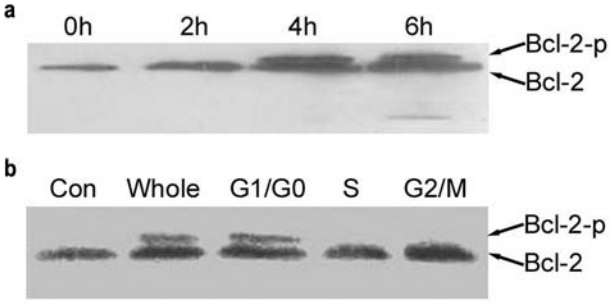

Figure 4. X-irradiation-induced increases in Bcl-2 phosphorylation in G1 phase MOLT-4 human lymphocytic leukemia cells. (*a*) MOLT-4 cells were exposed to X-irradiation (20 Gy), and cells were then harvested at different time points, as indicated. Electrophoresis was performed to separate phosphorylated Bcl-2 (Bcl-2-p) from Bcl-2 (Bcl-2). As seen, the levels of Bcl-2-p in intact cells are increased after X-ray exposure in a time-dependent manner. (*b*) MOLT-4 cells were exposed to X-ray (20 Gy) for 4–6 h, and the non-irradiated cells were used as controls. Irradiated cells were divided into four groups (whole cells, G1/G0, S, and G2/M) by flow sorting. Western blot analysis of cellular lysates from the sorted cells shows increased Bcl-2 phosphorylation (Bcl-2-p) in the G1/G0 phase cells.

(G1 cell cycle arrest) and in delivering anti-apoptotic signaling. PI3K activates intracellular serine/threonine AKT. Activated AKT acts through inhibition of the activation of the caspases and phosphorylation of the Bcl-2 family member, Bad, causing its dissociation from Bcl-X_L, which in turn suppresses cell death, while PTEN (a tumor suppressor protein with phosphatase activity) blocks PI3K activity by preventing activation of AKT. Expression of wild-type PTEN in tumor cells containing endogenous mutant PTEN inhibits AKT activity, induces G1 cell cycle arrest, and causes apoptosis. Evidence is accumulating that inhibition of PI3K with PI3K inhibitors causes G1 growth arrest and apoptosis in different carcinoma cells and normal cells [32–35]. A recent study reported that X-radiation in combination with the PI3K inhibitor LY294002 potentiated radiation-induced human esophageal cancer cell-killing synergistically, indicating that selective inhibition of the PI3K survival signaling pathway enhanced radiosensitivity in these cells [36]. These observations demonstrate that PI3K activity is required for progression through the G1 phase and that, in the absence of PI3K activity, cells are induced for apoptosis in this particular phase of the cell cycle, and further suggest that the PI3K/AKT pathway may also be involved in X-ray-induced G1 cell cycle arrest and apoptotic cell death in lymphocyte leukemia and other cells. However, the role and mechanism of the PI3K/AKT/PTEN pathway in this process remain elusive. It is possible that X-irradiation may down-regulate PI3K activity either directly by reducing intracellular 3′ phosphoinositide levels or indirectly by inhibiting AKT activity through PTEN induction in MOLT-4 cells. Nevertheless, the molecular basis for the signaling through the PI3K/AKT pathway, in regulating X-ray-induced G1 growth arrest and apoptosis in MOLT-4 cells, remains to be experimentally determined.

While many questions remain unanswered, it is clear that a specific sequence of cellular events appears to take place after exposure to X-irradiation. Some of these events may greatly influence the activity of the apoptosis pathway leading to MOLT-4 cell death. Based on the observations in this study, we propose the following hypothesis for genotoxic stress-induced apoptosis via modulation of cyclin B1-CDK1 activity in G1 MOLT-4 cells. In this model, DNA damage by X-rays produces decreased CDK2 activity and increased threshold levels of cyclin E through the p53-p21 pathway and/or the Chk-CDC25 pathway, thereby resulting in cell cycle arrest at the G1 phase. Cell cycle arrest leads to increased cyclin B1 levels in G1 cells, likely due to up-regulation of cyclin B1 transcription and inhibition of ubiquitination and proteolysis. This unscheduled mode of cyclin B1 serves as a modulator for CDK1 activation in G1 phase. CDK1 is also activated through dephosphorylation of Thr-14 and Tyr-15 (Fig. 2c) resulting from dual phosphatase CDC25C activity, as well as phosphorylation on Thr-161 (Fig. 2c) by CDK-activating kinase (a heterodimer of CDK7 and cyclin H). Once active, the CDK1-cyclin B1 complex phosphorylates CDC25C to enhance its phosphatase activity, thereby producing a positive feedback loop. It is at this stage that the CDK1 activity and level of cyclin B1 needed for activating CDK1 in the G1 phase might determine the length of G1 arrest necessary for DNA damage repair. Ultimately, prolonged G1 arrest would lead to apoptosis to eliminate the injured cells. Our data suggest that the phosphorylation of Bcl-2 (Fig. 4) may be a downstream event in the intrinsic apoptosis pathway, following CDK1 activation in G1, which finally executes cell death due to the loss of its ability to prevent caspase activation [37].

In conclusion, the present study documents an important role for CDK1 (CDC2) in regulation of apoptosis in X-irradiated MOLT-4 cells, in addition to its crucial roles in cell cycle control and other biological processes. Investigations are under way to further study how CDK1 serves a dual role in coordinately regulating cell growth and cell death under certain circumstances, and to elucidate the molecular basis for the signaling pathway(s) through which the unscheduled type of CDK1 regulates cell cycle control and apoptosis in different tumor and normal cells.

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