Lidamycin induces marked G2 cell cycle arrest in human colon carcinoma HT-29 cells through activation of p38 MAPK pathway

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Abstract. Lidamycin (LDM), a member of the enediyne antibiotic family, is presently undergoing phase I clinical trials in P.R. China. In this study, we investigated the mechanisms of LDM-induced cell cycle arrest in order to support its use in clinical cancer therapy. Using human colon carcinoma HT-29 cells, we observed that LDM induced G2 cell cycle arrest in a time- and dose-dependent manner. LDM-induced G2 arrest was associated with increasing phosphorylation of Chk1, Chk2, Cdc25C, Cdc2 and expression of Cdc2 and cyclin B1. In addition, cytoplasmic localization of cyclin B1 was also involved in LDM-induced G2 arrest. Moreover, we found that p38 MAPK pathway contributed to LDM-induced G2 arrest. Inhibition of p38 MAPK by its inhibitor SB203580 not only attenuated LDM-induced G2 arrest but also potentiated LDM-induced apoptosis, which was accompanied by decreasing phosphorylation of Cdc2 and increasing expression of FasL and phosphorylation of JNK. Finally, we demonstrated that cells at G1 phase were more sensitive to LDM. Together, our findings suggest that p38 MAPK signaling pathway is involved in LDM-induced G2 arrest, at least partly, and a combination of LDM with p38 MAPK inhibitor may represent a new strategy for human colon cancer therapy.

Introduction

Cell cycle arrest and apoptosis are common phenomena after DNA-damaging treatment (1-3). In response to DNA damage, ATM and ATR are activated, and then ATM phosphorylates Chk2 on Thr-68 leading to Chk2 kinase activation (4), while both ATM and ATR phosphorylate Chk1 on Ser-317 and Ser-345 resulting in its activation (5,6). Activated Chk1 and Chk2 phosphorylate Cdc25C on Ser-216, which promotes the interaction of Cdc25C with 14-3-3 proteins and inhibits the ability of Cdc25C to activate Cdc2/cyclin B1, resulting in G2 cell cycle arrest (7). In addition, several lines of evidence demonstrated that constitutive nuclear accumulation of cyclin B1 caused a reduction in damage-induced G2/M arrest, thereby raising the possibility that DNA-damage signaling acts in part by stabilizing the cytoplasmic localization of cyclin B1 (8,9).

Recently, it has been demonstrated that other pathways contribute to the G2/M checkpoint, among which include MAPK cascades. In mammals, MAPKs are divided into three major groups, p38 MAPK, JNK and ERK. Since Bulavin *et al* found that p38 MAPK was required for ultraviolet radiation induced G2/M arrest (10), the role of p38 MAPK in G2/M cell cycle checkpoint has been confirmed by other studies (11,12). Accumulating evidence also indicates that ERK has been implicated in G2/M checkpoint (13,14). In contrast, several studies have demonstrated that inhibition of JNK can induce G2/M arrest (15,16). Furthermore, recent data suggest that MAPKs may mediate apoptotic signaling induced by anticancer drugs. However, these MAPKs have been shown to exert both antiapoptotic and proapoptotic effects in different studies. For example, p38 MAPK activation is a critical pathway in glucocorticoid-induced cell death (17), whereas inhibition of p38 can potentiate sulindac sulfide induced apoptosis in colon carcinoma cells (18). Together, these results indicate that the relationship of MAPKs with cell cycle arrest and apoptosis is complicated and may be dependent on the cellular context, cell type and stimuli.

Lidamycin (LDM, originally named C-1027) is a member of the enediyne antibiotic family, which was isolated from a *Streptomyces globisporus* C1027 strain (19). As a DNA-damaging agent, LDM is characterized by inducing a higher ratio of DNA double-strand breaks (DSBs) than single-strand breaks (SSBs) (20). It is well known that DSB is the most severe DNA lesion, which may explain the highly potent cytotoxicity of LDM towards cancer cells. It has been reported that LDM can induce cell cycle arrest (21), and LDM-induced cell cycle arrest was dependent on drug concentration and p53 status (22). However, the detailed molecular mechanisms and signaling pathways of LDM-induced cell cycle arrest are not well understood.

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Abbreviations: LDM, lidamycin; PI, propidium iodide; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; ERK, extracellular signal regulated kinase; DMSO, dimethyl sulfoxide; ATM, ataxia-telangiectasia-mutated; ATR, ATM- and Rad-3-related protein kinase; FITC, fluorescein isothiocyanate; PARP, poly(ADP-ribose) polymerase

Key words: lidamycin, G2 arrest, MAPK, HT-29 cells

In the present study, we intended to further elucidate the molecular mechanisms of LDM-induced cell cycle arrest in HT-29 cells. Specifically, we examined the influence of p38 MAPK signaling upon LDM-induced G2 arrest and cell death. Finally, we investigated whether the sensitivity of HT-29 cells to LDM was cell cycle dependent.

Materials and methods

Cell culture and chemicals. Human colon carcinoma HT-29 cells were cultured in DMEM (Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemical Co., St. Louis, Missouri), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37˚C in a humidified atmosphere containing 5% CO_2.

LDM was generously provided by Professor Jin (Institute of Medicinal Biotechnology, CAMS). LDM stock solution (10 μM) was prepared in water and stored at -70˚C. SB203580 was purchased from Calbiochem (San Diego, CA) and dissolved in DMSO (Sigma). All other chemicals were purchased from Sigma.

Cell synchronization. HT-29 cells were synchronized as described previously with a little modification (23). Briefly, the cells were synchronized at the G1 phase by placing unsynchronized cells in the serum-free medium for 48 h and then replacing medium containing 10% serum for 3 h. To obtain cells synchronized at the S phase, the cells were treated with 5 μg/ml aphidicolin for 24 h, and then washed and maintained in the drug-free medium for 3 h, while maintaining cells in the drug-free medium for 9 h to obtain the G2/M phase cells. Mitotic cells were synchronized by incubating cells with 0.4 μg/ml nocodazole for 24 h.

Drug treatment. For all treatment of LDM, cells were exposed to LDM as described below in each experiment. For treatment of SB203580, cells were pretreated with 20-μM SB203580 for 1 h, after which cells were exposed to LDM for 48 h in the presence of SB203580. The final DMSO concentration did not exceed 0.1% (vol/vol).

Antibodies. Cyclin B1 (GNS1), cyclin A (H-432), Cdc2 (C-19), p21 (F-5), actin (I-19), rabbit p38 MAPK (C-20), JNK1 (C-17) and PARP (F-2) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Chk1 (2345), Chk2 (2662), phospho-Cdc2 (Tyr-15) (9111), phospho-Chk1 (Ser-345) (2341), phospho-Chk2 (Thr-68) (2661), phospho-Cdc25C (Ser 216) (9528), phospho-p38 MAP kinase (Thr180/Tyr182) (9211), phospho-SAPK/JNK MAP kinase (Thr-183/Tyr-185) (9251) and phospho-ATF2 (Thr-71) (9221) antibodies were from Cell Signaling Technology (Beverly, MA). FasL (Ab-1) antibody was purchased from Calbiochem.

Flow cytometry analysis. Floating and attached cells were collected. Cells were fixed in ice-cold 70% ethanol and stored at -20˚C. Samples were then washed twice in PBS and resuspended in a solution of PI (50 μg/ml) and RNase A (0.5 mg/ml) in PBS for 30 min at 37˚C in the dark. The stained cells were filtered through 40 μm gauze, and the single-cell suspensions were analyzed on an Epics-XL flow cytometer (Coulter Electronics, Inc., Miami, FL) using Mcycle software.

In MPM-2/PI bivariate flow cytometry, cells were labeled with MPM-2 antibody (final concentration of 1 μg of MPM-2 antibody/ml) for 1 h at 4˚C. Cells were washed once with PBS and incubated with antimouse IgG conjugated with FITC antibody (Santa Cruz Biotechnology) for 1 h at room temperature in the dark. After washing with PBS, cells were resuspended in 5 μg/ml PI containing 50 μg/ml RNase A for 30 min in the dark (24). Samples were analyzed on an Epics-XL flow cytometer. The MPM-2 positive cells (mitotic cells) showed increased green fluorescence, thus shifting above the baseline of the dot plot.

Western blotting. The procedure has been described previously (22). Briefly, cells were harvested, and washed with PBS solution. The whole cellular extracts were prepared by incubating cells on ice in lysis buffer. The cell lysates were cleared by centrifugation at 12,000 x g for 12 min. Protein concentrations were determined by Bradford assay. Equal amounts of lysate (40 μg) were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA). Membranes were blocked in TBST containing 5% nonfat skim milk at room temperature for 2 h and probed with primary antibodies overnight at 4˚C. Then membranes were blotted with an appropriate horseradish peroxidase-linked secondary antibody (Santa Cruz Biotechnology). Proteins were visualized using enhanced chemiluminescence Western blotting detection reagents (Amersham Pharmacia Biotech, Inc., Piscataway, NJ).

Indirect immunofluorescence. Cells were grown on sterile coverslips and treated with drugs for an indicated time. Then cells were fixed with 3.7% formaldehyde in PBS for 10 min at 37˚C and permeabilized in 0.2% Triton-100 in PBS for 10 min at room temperature. Nonspecific binding sites were blocked by incubating the cells with 3% bovine serum albumin in PBS for 1 h at 37˚C, and stained with mouse monoclonal anti-cyclin B1 antibody overnight at 4˚C as described previously (8). After washing 3 times with PBS, cells were stained with FITC-labelled anti-rabbit IgG antibody for 1 h at room temperature in the dark. After incubation, the coverslips were washed with PBS, and imaged using an Olympus IX-70 inverted fluorescent microscope (Olympus, Tokyo, Japan).

Hoechst 33342 staining. Cells were treated with LDM or co-treated with LDM and SB203580 for 48 h, and then the floating and attached cells were collected. After being centrifuged and washed with PBS, cells were fixed with 4% paraformaldehyde for 10 min at room temperature and then washed once with PBS. Hoechst 33342 (5 μg/ml) was added to the fixed cells, incubated for 10 min at room temperature. Then cells were spread on coverslips, and imaged using an Olympus IX-70 inverted fluorescent microscope. For each determination, at least 300 cells were counted from more than 5 random microscopic fields. The percentage of apoptotic death cells was calculated from the number of cells with apoptotic (condensed and fragmented nuclei) nuclear morphology divided by the total number of cells examined.

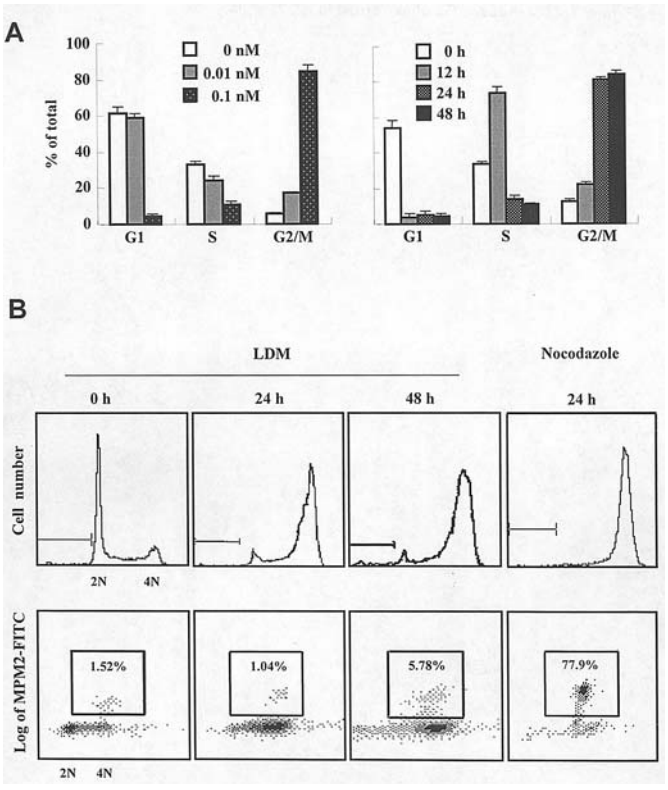

Figure 1. LDM induces G2 phase arrest in HT-29 cells. (A) PI staining for cell cycle distribution. Cells were treated with LDM for 48 h at the indicated concentrations (left panel) or treated with 0.1 nM LDM for the indicated time (right panel), and then the cell cycle distribution was determined by flow cytometry after PI staining. Error bars represent means ± SD. (B) The MPM-2/PI bivariate flow cytometry for the MPM-2 expression. Cells were treated with 0.1 nM LDM at the indicated times as described in 'Materials and methods'. Cells treated with 0.4 μg/ml nocodazole for 24 h served as a positive control. Mitotic cells are indicated by a box in the dot plots. All data are representative of three independent experiments.

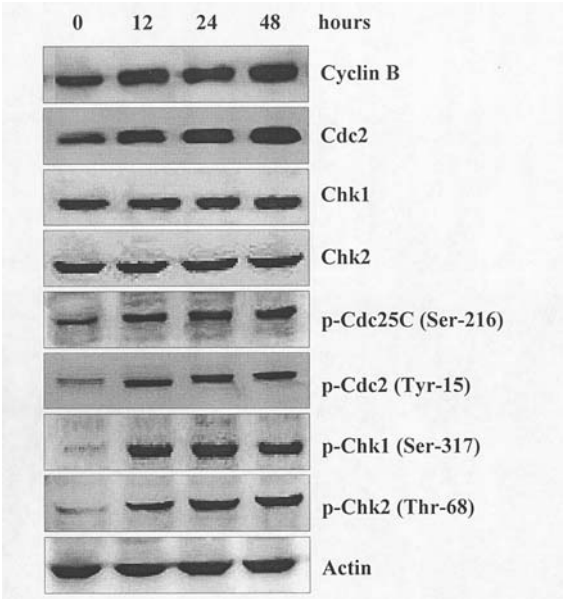

Figure 2. Changes of cell cycle related proteins in LDM-treated HT-29 cells. Cells were treated with 0.1 nM LDM for the indicated time. Cell lysates were prepared for SDS-PAGE and analyzed by Western blotting as described in 'Materials and methods'. Actin was used as a loading control. Data are representative of three independent experiments.

Results

LDM induces G2 arrest in HT-29 cells. First, we determined the cell cycle alteration after LDM treatment in HT-29 cells. Cells were labeled with PI and analyzed by DNA flow cytometry. After treatment with LDM, the cells significantly accumulated in the G2/M phase in both a time- and concentration-dependent manner (Fig. 1A). At 1 nM, the apoptotic cells were markedly increased, as detected in a prominent sub-G1 peak (data not shown). For this study, we chose 0.1 nM for the subsequent assays before cells underwent apoptosis.

Conventional DNA flow cytometry does not distinguish cells in the G2 phase from cells in the M phase, because cells in both phases have the same DNA content (25). Therefore, we used an MPM-2 antibody that reacted specifically with phosphoprotein presented in M phase cells in combination with PI staining to further analyze the effects of LDM on cell cycle progression (26). After cells were treated with 0.4 μg/ml nocodazole (a known agent inducing M arrest) for 24 h, 77.9% of cells were MPM-2 positive (mitotic cells), indicating that most cells were in M phase which validated the method (Fig. 1B). In contrast, although the result of PI staining indicated that >80% of cells were accumulated in G2/M phase when treated with 0.1 nM LDM for 24 h (Fig. 1A), only 1.04% of cells were MPM-2 positive (Fig. 1B), indicating that most cells were arrested in G2 phase. Although the mitotic cells were slightly increased at 48 h (from 1.04% to 5.78%), most of the cells were MPM-2 negative, suggesting cells were still mainly accumulated in G2 phase.

Changes of cell cycle related proteins by LDM. Based on the above results, we next investigated the effect of LDM on G2 phase-associated regulatory molecules. The data showed that LDM treatment resulted in an increase in the protein expression of cyclin B1 and Cdc2 (Fig. 2). In addition, the levels of phosphorylated Cdc2 (Tyr-15) and phosphorylated Cdc25C (Ser-216) were increased by LDM (Fig. 2). Although total protein levels of Chk1 and Chk2 were unchanged, activated forms of Chk1 (phosphorylated at Ser-317) and Chk2 (phosphorylated at Thr-68) were obviously increased after LDM treatment (Fig. 2), indicating that both Chk1 and Chk2 pathways were activated by LDM.

It has been reported that p21 plays a role in inducing the G2 arrest and expression of p21 can be induced in a p53-independent pathway (27,28). Thus, we investigated whether LDM can induce p21 expression in p53 mutant HT-29 cells. After LDM treatment, the protein levels of p21 were undetected in both control and treated HT-29 cells (data not shown), indicating that LDM-induced G2 arrest was independent of p21 and LDM cannot induce p21 expression in p53 mutant HT-29 cells.

Cytoplasmic localization of cyclin B1 in LDM-treated cells. Since cytoplasmic localization of cyclin B1 has been implicated in establishing G2 arrest after DNA damage (8,9), we examined the subcellular localization of cyclin B1. We found that cyclin B1 mostly accumulated in the cytoplasm after LDM

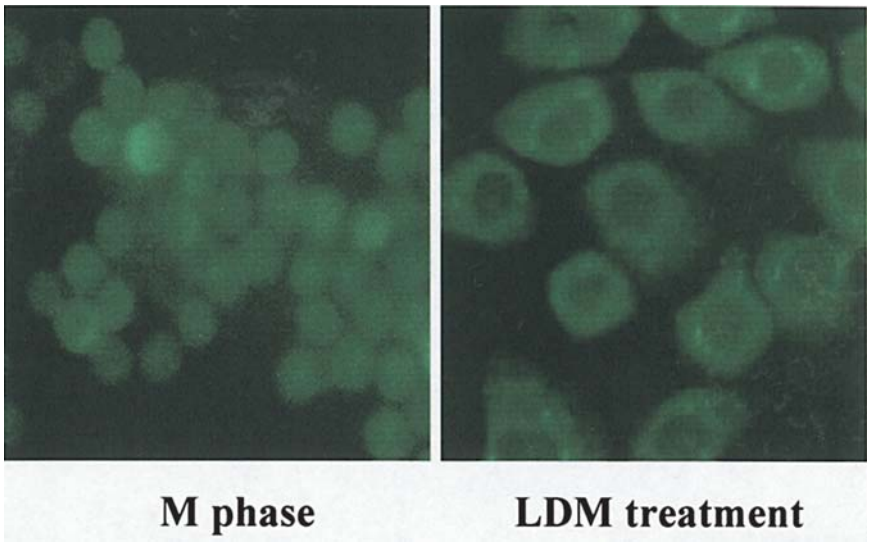

Figure 3. Cytoplasmic localization of cyclin B1 after LDM treatment. HT-29 cells were treated with 0.1 nM LDM for 24 h, and then fixed and stained as described in 'Materials and methods'. Mitosis phase synchronized cells were used as control. Green fluorescence indicates the localization of cyclin B1. Data are representative of two independent experiments.

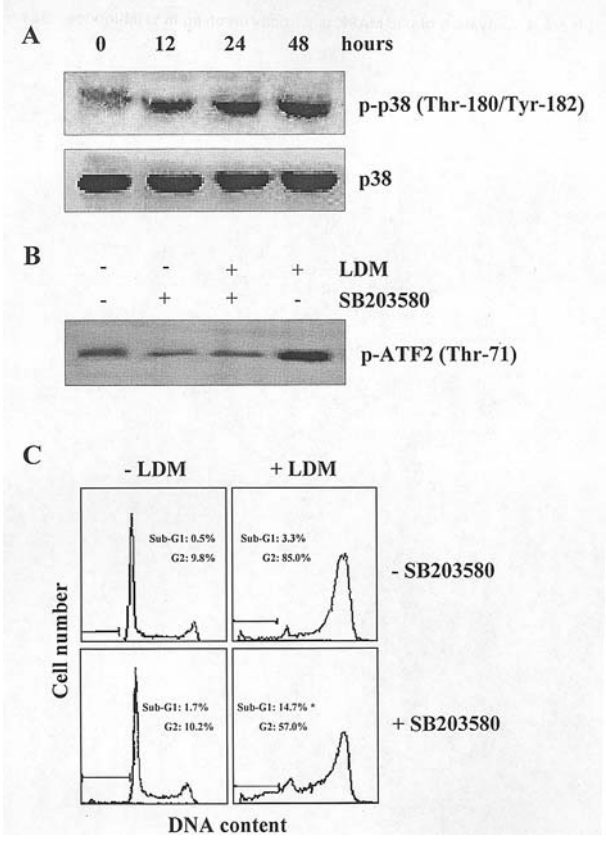

Figure 4. Activation of p38 MAPK is partially involved in LDM-induced G2 arrest. (A) Cells were treated with 0.1 nM LDM for the indicated time, and then the phospho-p38 MAPK expression was assessed by Western blotting using phospho-specific antibody. (B) Cells were treated with 0.1 nM LDM in the presence or absence of 20 μM SB203580 for 48 h. The p38 MAPK activity was assessed by Western blotting using phospho-ATF2 (the p38 substrate) antibody. (C) Cells were treated with 0.1 nM LDM in the presence or absence of 20 μM SB203580 for 48 h, and then analyzed by flow cytometry after PI staining. All data are representative of three independent experiments. $^{*}p<0.05$, compared with LDM treatment alone.

treatment, whereas in cells synchronized at M phase, cyclin B1 was mainly localized in the nucleus (Fig. 3). These data suggest that cytoplasmic localization of cyclin B1 also contributes to LDM-induced G2 arrest.

Activation of p38 MAPK is involved in LDM-induced G2 arrest. Recent reports suggested that p38 MAPK signaling pathway might mediate G2 cell cycle arrest (10-12). Therefore, we tested the effect of LDM on p38 MAPK using phospho-specific antibody (its activation form). As shown in Fig. 4A, exposure of HT-29 cells to 0.1 nM LDM resulted in significant and sustained activation of p38 MAPK. To further study whether p38 MAPK activation is associated with LDM-induced G2 arrest, we used SB203580, a specific p38 MAPK inhibitor, to inhibit the p38 activation. As shown in Fig. 4B, phosphorylation of the transcription factor ATF2, a downstream target of p38 MAPK, was markedly increased after LDM treatment, which supported the result that p38 MAPK pathway was activated in LDM-treated HT-29 cells. However, in the presence of SB203580, LDM-induced ATF2 phosphorylation was obviously decreased compared with LDM treatment alone (Fig. 4B), suggesting that activation of p38 MAPK induced by LDM was inhibited by SB203580.

Subsequently, to evaluate the contribution of the p38 MAPK activation to LDM-induced G2 arrest, we investigated the cell cycle alteration of LDM-treated HT-29 cells in the presence and absence of SB203580 by flow cytometry. As shown in Fig. 4C, pretreatment with SB203580 for 1 h attenuated the LDM-induced G2 population accumulation (decrease from 85% to 57%), but could not completely abolish the G2 arrest. Taken together, these results suggest that p38 MAPK pathway partially mediates the LDM-induced G2 arrest.

Inhibition of p38 MAPK decreases the phosphorylated level of Cdc2. To further investigate the mechanism by which

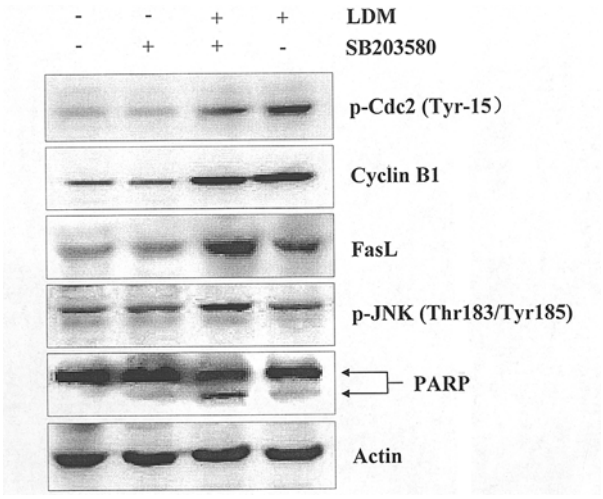

Figure 5. Effects of SB203580 on Cdc2 and JNK phosphorylation, and FasL expression. Cells were treated with 0.1 nM LDM in the presence or absence of 20 μM SB203580 for 48 h. Cell lysates were prepared for SDS-PAGE and analyzed by Western blotting as described in 'Materials and methods'. Actin was used as a loading control. Data are representative of three independent experiments.

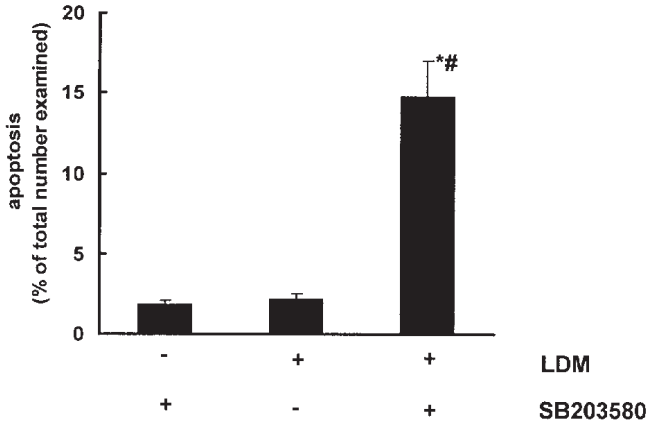

Figure 6. SB203580 potentiates LDM-induced apoptosis. Cells were treated with 0.1 nM LDM in the presence or absence of 20 μM SB203580 for 48 h, then imaged using an inverted fluorescent microscope as described in 'Materials and methods'. The percentage of apoptotic death cells was calculated from the number of cells with apoptotic nuclear morphology divided by the total number of cells examined. Data are representative of three independent experiments. Original magnification, x200. $^{*}p<0.05$, compared with SB treatment alone. $^{\#}p<0.05$, compared with LDM treatment alone.

inhibition of p38 MAPK can attenuate LDM-induced G2 arrest, we tested the expression of cyclin B1 and the phosphorylated level of Cdc2 in the presence and absence of SB203580. We found that pretreatment with SB203580 for 1 h decreased the phosphorylated levels of Cdc2 but had no influence on cyclin B1 expression compared with LDM treatment alone (Fig. 5). These data suggest that inhibition of p38 MAPK can protect Cdc2 from inactivation by LDM, thereby reducing the LDM-induced G2 arrest.

Inhibition of p38 MAPK potentiates cell death induced by LDM. Unexpectedly, we found that pretreatment with SB203580 for 1 h not only reduced the LDM-induced G2 arrest, but also increased the sub-G1 population compared with LDM treatment alone (Fig. 4C, $p<0.05$), implying that cell death induced by LDM was increased after inhibition of p38 MAPK. This result was confirmed by Hoechst 33342 staining (Fig. 6). In comparison with LDM treatment alone, the percentage of cells with apoptotic nuclear morphology was significantly increased in SB203580 and LDM co-treated cells (Fig. 6B, $p<0.05$). Moreover, the cleavage fragment of PARP was also increased in LDM and SB203580 co-treated cells (Fig. 5), supporting the result that inhibition of p38 MAPK can enhance LDM-induced cell death in HT-29 cells.

Inhibition of p38 MAPK increases the levels of phosphorylated JNK and FasL. Next, we investigated the mechanism by which pretreatment with SB203580 potentiated the LDM-induced cell death. In the apoptosis-related molecules, we found that only the expression of FasL was increased in SB203580 and LDM co-treated cells compared with LDM treatment alone (Fig. 5), whereas the expression of Bcl-2 and Bax was unchanged (data not shown), indicating that inhibition of p38 MAPK might activate the FasL/Fas apoptosis pathway in LDM-treated cells, thereby leading to an enhanced susceptibility of cells to LDM-induced cell death. Surprisingly, we found that although the activation (phosphorylated) of JNK was unchanged in LDM-treated HT-29 cells, the combination of LDM and SB203580 markedly increased the phosphorylated level of JNK compared with LDM single treatment (Fig. 5), suggesting JNK was activated in LDM and SB203590 co-treated cells.

Cells at G1 phase are sensitive to LDM. We also investigated whether the sensitivity of HT-29 cells to LDM is cell cycle dependent. Synchronized cells (Fig. 7A) were released by replacing medium containing 10% serum at different cell cycle phases, and then treating with 0.1 nM LDM for 48 h. Cells were collected and analyzed by flow cytometry. Results showed that cells synchronized at G1 phase are more sensitive to LDM compared with cells synchronized at S and G2/M (Fig. 7B), and the sub-G1 populations are 22.4%, 10.8% and 3.9%, respectively. These data imply that LDM may be a cell cycle-dependent DNA damaging agent.

Discussion

LDM is currently being evaluated in clinical trials as a potential anticancer agent in P.R. China. Our previous study showed that LDM-induced different cell cycle arrests were dependent on cell lines (22). In this study, we focused on the mechanisms and signaling pathways of LDM-induced cell cycle arrest in HT-29 cells. Using PI staining, the data showed that LDM induced cell cycle arrest in G2/M phase. We further confirmed that LDM principally induced G2 phase arrest by MPM-2/PI bivariate flow cytometry. The data suggest that LDM induces distinctly G2 arrest in p53 mutant human colon cancer cells.

Chk1 and Chk2 are key elements of the DNA damage-induced G2 checkpoint. Chk1 and Chk2 are activated in response to DNA damage and phosphorylate Cdc25C on serine-216. Phosphorylated Cdc25C is then complexed with

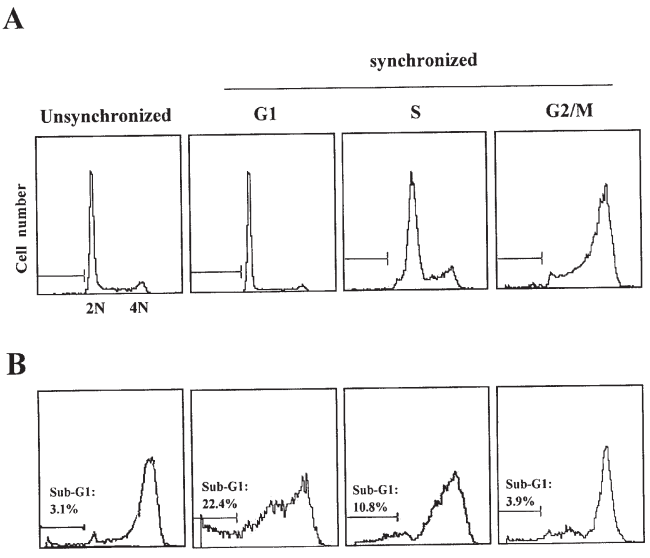

Figure 7. Cells at G1 phase are more sensitive to LDM. Cells were synchronized as described in 'Materials and methods'. Then cells were released by replacing medium containing 10% serum with 0.1 nM LDM for 48 h. Cells were collected and analyzed by flow cytometry after PI staining. Unsynchronized cells were served as a control and the sub-G1 population represents the dead cells. Data are representative of three independent experiments.

14-3-3 protein, and inhibits the ability of Cdc25C to activate Cdc2/cyclin B1, resulting in G2 cell cycle arrest (7). We found that phosphorylation of Chk1 and Chk2 was increased after LDM treatment, which was consistent with our previous study (22). Because these phosphorylated levels represented their activation forms, these data indicated that both Chk1 and Chk2 pathways were activated by LDM. Furthermore, both phosphorylated Cdc25C and phosphorylated Cdc2 were also increased, which confirmed activation of Chk1 and Chk2. Regarding the increases of cyclin B1 and Cdc2 proteins, these changes may be related with the increase in proportion of cells at G2 phase, and are not associated with kinase activity, which is consistent with a previous report (29). These data indicated that Chk1 and Chk2 negatively regulated Cdc2 kinase by phosphorylating on tyrosine-15 in LDM-treated cells. Thus, we propose that DNA damage induced by LDM activates Chk1 and Chk2 kinases guarding against mitotic entry from G2 phase.

The biological function of Cdc2/cyclin B1 is regulated by changes in subcellular localization. Some reports have demonstrated that G2 cell cycle arrest is further assured by stabilizing the cytoplasmic localization of cyclin B1 (8,9,30,31). Fig. 3 shows that cyclin B1 accumulated in the cytoplasm in LDM-treated cells, supporting that cytoplasmic localization of cyclin B1 could contribute to G2 arrest induced by LDM in HT-29 cells. So far, we cannot determine how LDM causes cytoplasmic localization of cyclin B1. It is necessary in further studies to confirm whether LDM promotes nuclear export of cyclin B1 or inhibits its import into the nucleus.

It has been reported that p38 MAPK is involved in G2 checkpoint pathway. Activation of p38 MAPK is required for the γ-radiation-induced G2 arrest (32) and p38 MAPK has a crucial role in the initiation of G2/M checkpoint in response to UV radiation (10). Consistent with this notion, our results showed that p38 MAPK was activated in LDM-treated cells. Furthermore, we found that inhibition of p38 MAPK by SB203580 partially reduced the phosphorylated level of Cdc2 compared with LDM alone. It is well known that reduced activity of Cdc2 is essential for the cell cycle arrest at G2 phase (33). Thus, protection of Cdc2 inactivation by LDM may be the reason why inhibition of p38 MAPK can lead to attenuation of the G2 arrest, indicating that p38 MAPK pathway was associated with LDM-induced G2 arrest.

Cell cycle arrest may provide the time for damaged-DNA repairing, thereby increasing the chance of cell survival. This suggests that abolition of cell cycle arrest may enhance the sensitivity of cells to DNA damaging agents. The previous study indicated that cell cycle checkpoint abrogator UCN-01 enhances the cytotoxicity of DNA damaging agents in p53-deficient cells, which is consistent with this notion (34). In this study, we found that inhibition of p38 MAPK attenuated LDM-induced G2 arrest, and potentiated LDM-induced cell death. Our data raises the possibility that combination of LDM with p38 MAPK inhibitor may be a new strategy for colon cancer therapy.

Further study demonstrated that the increase in cell death might be related with increasing FasL expression. In addition, the JNK was activated in LDM and SB203580 co-treated cells (Fig. 5). A recent report has demonstrated that FasL is a target in the JNK/c-jun signaling pathway that induces apoptosis (35). We suppose that activation of JNK and subsequent increase of FasL by co-treatment with LDM and SB203580 are necessary for HT-29 cells undergoing apoptosis. Our data also indicated that LDM-induced cell death was dependent on cell cycle progression. Fig. 7 shows that the apoptotic sub-G1 peak was higher in cells synchronized at G1 phase than cells synchronized at S and G2/M phases. These results may imply that the sensitivity of cells to LDM is correlated with DNA synthesis.

In summary, our findings suggest that LDM induces distinctly G2 arrest in p53 mutant human colon carcinoma HT-29 cells. Moreover, this is the first report that p38 MAPK is associated with LDM-induced cell cycle arrest and cell death. Although there might be other mechanisms and signaling pathways to regulate the activity of LDM, the present study identifies at least a possibility that p38 MAPK may be a target for sensitizing colon cancer cells to LDM.

Acknowledgments

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