Actin disruption agents induce phosphorylation of histone H2AX in human breast adenocarcinoma MCF-7 cells

IK JAE SHIN[1], YONG-TAE AHN[3], YONGKUK KIM[4], JONG-MYOUNG KIM[5] and WON G. AN[1,2]

[1]Joint Research Center of Pusan National University-Fraunhofer IGB; [2]School of Korean Medicine, Busan 609-735; [3]Department of Biomedical Laboratory Science, Kyungdong University, San 91-1 Bongpo-ri, Toseong-myeon, Goseong-gun, Gangwon-do 219-832; [4]Laboratory of Mathematical and Computational Biology, Department of Mathematics, Kyungpook National University, Daegu 702-701; [5]Department of Marine BioMaterials and Aquaculture, Pukyong National University, Busan 608-737, Republic of Korea

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Abstract. Modified actin dynamics are a unique feature of transformed cancer cells and thereby promising targets for cancer chemotherapy. While latrunculin B (LB) and pectenotoxin-2 (PTX-2), both derived from natural sources, inhibit actin polymerization, jasplakinolide (JSP) prevents actin depolymerization. The purpose of this study was to examine the detailed molecular action of actin disruption inducing apoptosis via double strand breaks (DSBs). Actin disruption induced phosphorylation of H2AX, a well known DSB marker leading to G2 arrest and consequently resulted in apoptosis on MCF-7 cancer cells. Cells impaired by actin disruption activated Erk (extracellular signal-related kinase) and p53 protein was involved in DNA damage responses, but did not change the levels of $p21^{Cip1/WAF1}$ protein in MCF-7 cells. To overcome the DSBs by actin disruption, MCF-7 cells set the repair system through the homologous recombination (HR) pathway. These results indicate that actin is involved in the signaling inducing DSBs and HR repair as well as G2 cell cycle arrest in human cancer. Therefore, the results suggest that actin disruption might be a potential candidate for developing anti-cancer therapies in human breast cancer.

Correspondence to: Dr Won G. An, School of Korean Medicine, Busan 609-735, Republic of Korea
E-mail: wgan@pusan.ac.kr

Abbreviations: ATM, ataxia telangiectasia mutated; BCA, bicinchoninic acid; BRCA1, breast cancer associated protein 1; DSB, double strand break; Erk, extracellular signal-related kinase; JSP, jasplakinolide; HR, homologous recombination; LB, latrunculin B; NHEJ, non-homologous end-joining; PTX-2, pectenotoxin-2; PIKKs, phosphatidylinositol-3 kinase-like kinases

Key words: actin cytoskeleton, latrunculin B, DNA double strand break, BRCA1, G2/M, p53, pectenotoxin-2

Introduction

Cells have to struggle to protect their genomic information from numerous environmental stresses during their lifetime. Among the genomic damage within the cell, DNA double-strand break (DSB) is probably the most dangerous. As soon as DSB is generated, histone H2AX is rapidly phosphorylated on serine 139 residues from the carboxyl-terminus to form γH2AX at the incipient sites of DSB by the phosphatidylinositol-3 kinase-like kinases (PIKKs) (1). One of the PIKKs, ataxia telangiectasia mutated (ATM) is a primary signal transducer in response to DSB and mediates the downstream signal proteins such as Chk1/2, p53, breast cancer associated protein 1 (BRCA1), and extracellular signal-related kinase (2,3). Serving as binding sites for repair proteins and chromatin remodeling complexes, γH2AX plays an important role for the response to conserve genomic stability (4,5). Cells have to operate two repairing system, homologous recombination and non-homologous end-joining (NHEJ), to renovate their damaged DSB. Two pathways are distinguished from the accuracy of repair and the requirement of homologous sequence, sister chromatid. HR is an error-free repair system but limiting to replicating cells in the S, G2/M phase of the cell cycle. In contrast, NHEJ is an error-prone system in all cell cycles of eukaryotes (6). HR is carried out by RAD52 epistasis group, which is implicated with a large number of proteins such as BRCA1, BRCA2, XRCC2 and XRCC3. BRCA1, a target of ATM, associated with BRCA2 and RAD51 forms complex which is scrutinizing the genome on the intact homologous sister chromatid and repairing the missing information on the broken strand without loss of genetic information. In NHEJ, Ku heterodimer binds to the DNA DSB ends and then DNA-PKcs forms a complex with Ku/DNA strands. This protects damaged DNA strands from nuclease digestion prior to ligation. Subsequently, the compromised DNA double stands are directly ligated together by the DNA ligase IV/XRCC4 complex (7). However, the precise mechanism of DSB repair is not completely elucidated and the components of these two systems are controversial (8).

As highly conserved during the evolution, actin is the most abundant and ubiquitous protein in eukaryotic cells. Actin dynamics in response to external and internal stimuli are responsible for cellular processes such as cellular membrane reorganization, motility, and cytokinesis. Previous studies revealed that the nuclear actin is involved in chromatin remodeling, mRNA transcription and transcription factor activity (9-11). Cancer cells generally were characterized by distinct changes e.g. alteration of nuclear/cytoplasmic ratio, alteration of cell adhesion, increase of motility and invasion, and disruption of cell division and apoptosis machineries. Since these unique morphological and phenotypical changes are known to be involved in the alteration of actin dynamics, understanding the mechanism of actin dynamics in cancer cells may allow a precise strategy to be formed (12,13).

Natural products disrupting the actin dynamics are thought to be potent anti-cancer agents, and thereby postulated to control cancer growth and proliferation. Actin polymerization is inhibited by swinholide A, latrunculin B (LB) and pectenotoxin-2 (PTX-2), in contrast to actin stabilizers, such as jasplakinolide (JSP) and phalloidin, disrupting actin depolymerization (14).

We searched the detailed molecular action of actin disruption on human breast cancer MCF-7 cells. Cancer cells treated with actin disrupting agents show apoptotic results including DSB, delaying the cell cycle at G2 and activation of p53 as well as ERK. The results indicate that actin disrupting agents could be a potential candidate for developing anti-cancer therapy in the human breast cancer cells.

Materials and methods

Cell culture, reagents and antibodies. Human breast cancer (MCF-7) cells were obtained from American Type Culture Collection (ATCC). Cells were grown in Dulbecco's modified Eagle's medium supplemented with 1% antibiotic-antimycotic (Gibco) and 10% fetal bovine serum (Gibco), and incubated at 37˚C in a humidified atmosphere of 5% CO_2. In order to address the cells to signals required for survival from actin disrupting agents, all experiment on DSB induction were carried out without serum and exogenous growth factors. Chemical reagents and antibodies were from following suppliers: latrunculin B (LB), jasplakinolide (JSP), anti-mouse p53 and BRCA1 monoclonal antibody from Calbiochem; pectenotoxin-2 (PTX-2) as a gift from Dr J.H. Jung, Pusan National University, Korea (15); propodium iodide, RNase A from Sigma; protease inhibitor cocktail tablets from Roche; nitrocellulose transfer membrane from Whatman; bicinchoninic acid (BCA) protein assay kit from Pierce (USA); CellTiter 96 Non-Radioactive Cell Proliferation Assay kit for MTT assay kit from Promega (USA); enhanced chemiluminescence (ECL) detection kit, IgG-specific monoclonal, IgG-polyclonal and FITC-monoclonal antibodies form GE Healthcare; anti-rabbit PARP, phosphor-Erk polyclonal antibodies from Cell Signaling Technology; anti-mouse phosphor-H2AX antibody (Ser139, clone JBW301) from Upstate; anti-mouse $p21^{WAF1/CIF1}$ antibody from BD transduction laboratories, anti-mouse DNA-PK antibody from Santa Cruz Biotechnology.

Viability assay. Cells/well (2×10^4) were incubated in 96-well flat bottom plates in 100 μl of medium and stabilized in a humidified atmosphere of 5% CO_2 at 37˚C for more than 12 h. Cells were exposed to LB (0.5, 1.5, 3.0 μM) and maintained for 24 h. The MTT assay was performed by manufacturer's instruction. The absorbance at 570 nm was determined using a multi plate reader (Perkin-Elmer, Model 1420 Victor 3™). Triplicate wells were assayed for each condition, the mean and standard deviations were determined. Mean ± SE were calculated and reported as the percentage of proliferation compared to control.

Immunocytochemistry. MCF-7 cells were grown on coverslip that were rinsed with cold PBS. For permeabilization, cells were incubated with 150 μl permeabilizing buffer (PB; 0.15 M NaCl, 10 mM Tris, 1 mM $MgCl_2$, 0.2 mM dithiothreitol (DTT), 0.5 mM $CaCl_2$, 25% glycerol, pH 8.0) (16) for 4-5 min; 150 μl PB + 0.5% Triton X-100 for 3.5 min; 150 μl PB for 30 min at 37˚C and washed with PBS-T. Cells were fixed in 4% paraformaldehyde (pH 7.2) for 15 min and washed twice with PBS-T (PBS + 0.2% Tween-20). Before cell staining, cells were blocked in PBS-T-S (PBS-T + 10% FBS) for 20 min. All antibodies were diluted in PBS-T-S. Cells were detected with primary DNA-PK (1:100), BRCA1 (1:100) for 1 h and washed with PBS-T. FITC-mouse second antibody (1:200) was incubated with rhodamine-phalloidin (1:500) and DAPI (0.5 mg/ml) for 60 min in the dark. After washing with PBS-T, the cells were observed under a fluorescent microscope (Olympus IX-71, Japan) and a confocal microscope (Olympus IX-81, Japan).

Cell extraction. Harvested cells were lysed in a total lysis buffer [protease inhibitor cocktail 1 tablet/50 ml TNES buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 2 mM EDTA, 100 mM NaCl)] on ice for 30 min. After centrifugation for 10 min at 12,000 rpm, the supernatant was stored at -70°C until its use. Acid extraction of histones was performed as follows. Cells were washed with PBS and resuspended in NETN buffer (150 mM NaCl, 1 mM EDTA pH 8.0, 20 mM Tris-HCl pH 8.0, 0.5% NP-40). After 10-min incubation on ice, nuclei were pelleted by centrifugation at 8,500 rpm for 5 min at 4°C. The pellet was resuspended in 0.1 M HCl and incubated for 10 min at room temperature. Acid-extracted histones were obtained by centrifugation at 8,500 rpm for 5 min at 4°C.

Western blot analysis. The protein concentration was determined by bicinchoninic acid (BCA, Pierce) protein assay according to the manufacturer's instructions. Total proteins (30 μg) were resolved by 8 or 12% SDS-PAGE gels, and then electrophoretically transferred onto a nitrocellulose transfer membrane (Whatman) by electroblotting. The membranes were soaked in TBS-T buffer [20 mM Tris, pH 7.4; 137 mM NaCl; 0.1% (v/v) Tween-20] containing 5% (w/v) non-fat milk, and were probed with the specific primary antibodies diluted in TBS-T. The proteins were then detected with the respective secondary antibody-linked horseradish peroxidase followed by enhancement of chemiluminescence (ECL, GE Healthcare). Ponceau-S (Sigma) staining of membranes and anti-actin was applied to confirm equal loading of the proteins and successful transfer to the membranes.

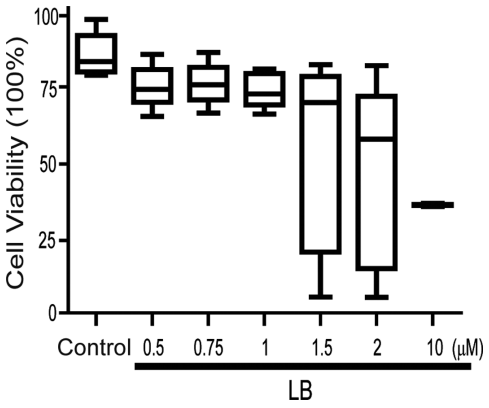

Figure 1. Effect of LB on the viability of MCF-7 cells. MCF-7 cells were treated with different concentrations of LB for 24 h, thereafter cell viability was determined by MTT assay. The values are represented as the percentage cell inhibition where untreated control cells were regarded as 100% (mean ± SE, n=12).

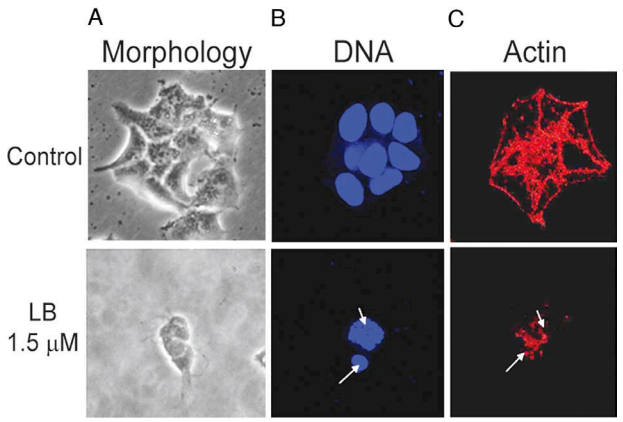

Figure 2. Disruption of actin microfilaments and induction of apoptosis by LB in MCF-7 cells. MCF-7 cells were treated with or without LB (1.5 μM) for 24 h. Phase contrast image (A) was taken by phase contrast and fluorescent microscope (Olympus IX-71, Japan). Cells were fixed with 4% paraformaldehyde and stained DNA with DAPI (B) or immunostained F-actin with rhodamine-phalloidine (C) using confocal microscope (Olympus IX-81, Japan). Each panel is representative of at least three experiments (×800).

Flow cytometry analysis for cell cycle and protein expression. For flow cytometry analysis, cells were treated with various concentrations of LB in a 6 well-plate in a humidified atmosphere of 5% CO_2 at 37°C for 6-24 h. Cells were washed with ice-cold PBS, and fixed in 100% ethanol at -20˚C for up to 2 weeks before the analysis of DNA contents or the expression level of γH2AX, DNA-PKcs and BRCA1. To analyze the cell cycle, cells were stained with propidium iodide (PI) solution containing 50 μg/ml PI, 0.1% nonidet P-40 (NP-40), 0.1% sodium citrate, and 100 μl/ml RNase A in the dark at 37°C for 30 min. To address LB-mediated protein expression, cells were stained with anti-phospho-histone H2AX (1:500), anti-DNA-PKcs (1:500) and anti-BRCA1 (1:500) antibodies at 37˚C for 1 h. Cells were incubated with secondary antibody (FITC-anti-mouse 1:500) for 1 h at room temperature. The percent of cells in each stage of the cell cycle and γH2AX, DNA-PKcs and BRCA1 protein expression were analyzed with FACSCalibur flow cytometry (BD Bioscience, France).

Results

LB induces cell growth inhibition in MCF-7 cells. The cytotoxicity of actin disrupting agent is a crucial obstacle in developing anticancer drugs based on actin. To designate a proper concentration of LB, the MTT assay was performed on MCF-7 breast cancer cells (Fig. 1). The viability of MCF-7 cells decreased in a dose-dependent manner upon treatment with various concentrations of LB (0.5-10 μM) for 24 h. The cell viability decreased rapidly from LB treatment at the concentration higher than 1.0 μM. The IC_{50} of LB was 7.1 μM for 24 h in MCF-7 cells. After being incubated with 1.5 and 10 μM LB for 24 h, cell viability was decreased by ~65.6 and 41.5% compared to that of control group, respectively.

LB destabilizes actin microfilaments and induces apoptosis in MCF-7 cells. In order to confirm the effect of LB on the morphology of human breast cancer cells, morphological feature and fluorescent image were examined by fluorescent and confocal microscopy. After 24 h of incubation with 1.5 μM of LB in MCF-7 cells, the morphological change of the cells was immediately investigated by phase contrast microscopy. Cell shape rapidly rounded with subcortical membrane following treatment with LB, although they maintained attachment to the plate. MCF-7 cells treated with LB gradually showed prominent morphological changes such as cytoplasmic shrinkage and marked convolution of cellular surface for 24 h (Fig. 2A). MCF-7 cells were exposed to 1.5 μM of LB for 24 h, and then assessed for morphological signs of apoptosis by staining with DAPI and for F-actin with rhodamine-phalloidin, then monitored by confocal microscopy. A distinguishing feature of apoptosis is the condensation and fragmentation of nuclear chromatin. Nuclear condensation and apoptotic bodies were observed in cells treated with LB (Fig. 2B). F-actin staining images showed that untreated control MCF-7 cells possessed a well-developed actin cytoskeleton with numerous subcortical actin filaments and stress fibers, while LB treated cells displayed slightly decreased F-actin and irregular aggregates of phalloidin-reactive material appeared in confocal microscopy images (Fig. 2C). These results elucidated that LB induced apoptosis through actin disruption in MCF-7 cells.

LB induces apoptosis in a time- and dose-dependent manner. In order to determine whether LB treatment induces apoptosis, the level of proteins involved in apoptotic signal transduction was examined by Western blotting. MCF-7 cells were treated with different concentrations of LB (0.1, 1.5 and 3 μM) for 24 h and with 1.5 μM of LB for 6, 12 or 24 h) (Fig. 3A and B). PARP cleavage was monitored with PARP antibodies by Western blot analysis, which specifically recognizes the 89-kDa fragment of the cleaved PARP and the uncleaved 116-kDa PARP. As shown in Fig. 3, the proportion of uncleaved 116-kDa PARP was decreased and the part of the 89-kDa cleavage product was increased with the treatment of LB in a time- and dose-dependent manner. However, cleaved 89-kDa PARP fragment except for the intact 116-kDa protein was not detected in the control cells (Fig. 3, lane 1). As shown

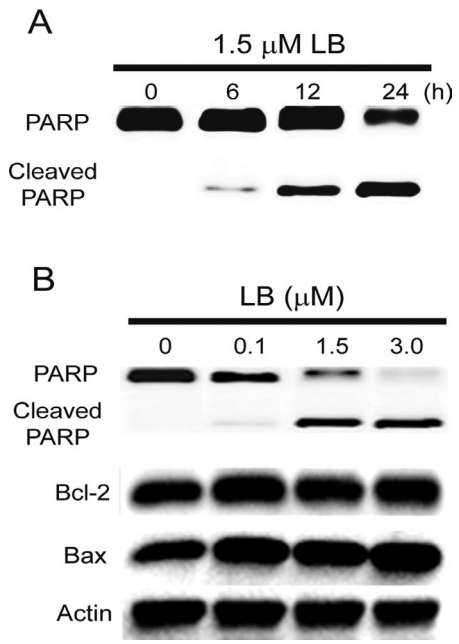

Figure 3. Effect of LB on the induction of PARP cleavage. MCF-7 cells were treated with 1.5 μM LB for 6, 12 and 24 h (A) and LB (0.1, 1.5 and 3.0 μM) for 24 h (B). Protein (30 μg) from cell total lysates was electrophoresed on SDS-PAGE, transferred to nitrocellulose, and probed with PARP, anti-Bcl-2, and anti-Bax antibodies respectively. Anti-actin was used for ensuring equal amount of protein loading.

in Fig. 3B, the level of Bax was slightly increased but Bcl-2 protein showed no change upon LB treatment in either cell line. The ratio of proapoptotic Bax and antiapoptotic Bcl-2 is known to be a barometer of apoptosis (17). Apoptotic signals activate Bax protein to reduce Bcl-2 in mitochondria, which is releasing the cytochrome c from mitochondria, supporting that actin disruption causes apoptosis through Bax signaling in MCF-7 cells.

LB induces double-strand breaks (DSB) in a time- and dose-dependent manner. DSB resulting from destructive DNA damage recruits phosphorylated H2AX in a broad damaged region at the early stage. Detection of γH2AX level is probably the most authentic method developed to date for measuring the DSB in cells. To investigate whether the apoptosis by LB is involved in DNA damage, especially for DSB, the level of γH2AX was measured by Western blotting and flow cytometry. As shown in Fig. 4, actin disruption introduced γH2AX to the DSB regions increasingly in a time- and dose-dependent manner in human cancer cells. The population of FITC-conjugated second antibody binding to γH2AX showed a time-dependent increase at 1.5 μM concentration of LB (13.3%, 12 h to 15.2%, 24 h) and dose-dependently in MCF-7 cells (Fig. 4A). The result was confirmed by Western blot analysis (Fig. 4B and C). Other actin disrupting agents, 0.25 μM JSP and 1 μM PTX-2, also recruited the γH2AX protein to protect from DNA damage (Fig. 4D). The results indicate that actin disruption induces DSB time- and dose-dependently in MCF-7 cells.

Actin disruption by LB arrests the cell cycle at G2 phase due to DNA damage. DSB is a severe genotoxic lesion as it leads to defective genomic integrity and genomic instability. Therefore, it is necessary to arrest the cell cycle to repair the lesions and help to maintain genomic stability. To assess whether LB induced DSB also prevents cells from transiting the cell cycle, MCF-7 cells treated with 1.5 μM LB for 12, 18 and 24 h were subjected to FACS analysis. The ratio of G2/M phase cells treated with 1.5 μM LB was 65.4% compared to 15.4% in the controls (Fig. 5A). The amount of phosphorylated Erk, one of the effectors of DNA damage gradually increased as the concentration of LB increased for 24 h in MCF-7 cancer cells (Fig. 5B). In response to a variety of stress damaging DNA including DSB, p53, a well known tumor suppressor, protects mammalian cells from tumor by inducing cell cycle

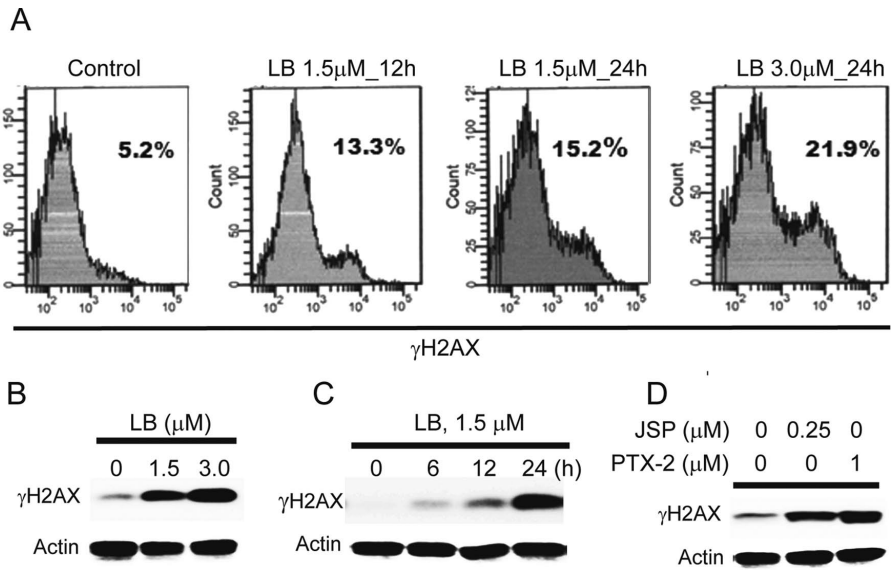

Figure 4. Effect of LB on the induction of DSB in MCF-7 cells. The γH2AX expression of MCF-7 cells treated with designated concentrations of LB and time were analyzed by flow cytometry (A) and Western blotting (B and C). The protein level of γH2AX in MCF-7 cells treated with other actin disrupting agent, JSP 0.25 μM or PTX-2 1 μM for 24 h is shown by Western blotting (D). Protein (5 μg) from cell acid lysates was electrophoresed on SDS-PAGE, transferred to nitrocellulose, and probed with γH2AX antibodies. Anti-actin was used to ensure equal amount of protein loading.

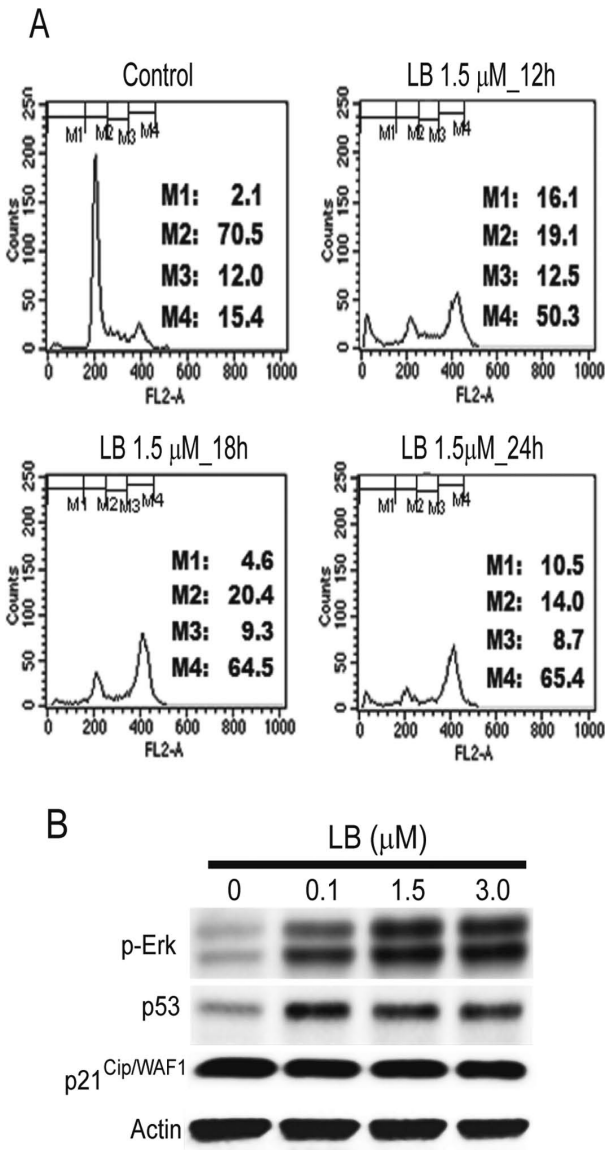

Figure 5. Effect of LB on the cell cycle progression and signal transduction in MCF-7 cells. (A) The cell cycle of MCF-7 treated with 1.5 μM LB for 12, 18 and 24 h were analyzed by flow cytometry. Control indicates the cells subjected to the same treatment without LB. DNA was stained with PI and the contents of DNA were determined by flow cytometry. Numbers indicate the percentage of cells in the phase of cell cycle (M1, G0; M2, G1; M3, S; M4, G2). (B) The signal transduction of MCF-7 treated with various concentrations of LB (0.5, 1.5 and 1.5 μM) was analyzed by Western blotting. Protein (30 μg) from cell total lysates was electrophoresed on SDS-PAGE, transferred to nitrocellulose, and probed with phospho-Erk, anti-p53, p21$^{Cip1/WAF1}$ antibodies, respectively. Anti-actin was used to ensure equal amount of protein loading.

arrest, DNA repair and apoptosis. p53 plays a pivotal role in G2 cell cycle arrest and DSB repair (18,19). As shown in Fig. 4B, p53 protein was stabilized by LB treatment for 24 h in MCF-7 cells. However, LB did not affect the level of p21$^{Cip1/WAF1}$, a p53 downstream effector and cdk inhibitor, in MCF-7 cancer cells (Fig. 4B). We also investigated the pathway of DSB repair evoked by LB.

DSB by actin disruption is repaired through the HR pathway. In response to DSB, cells activate the component protein of HR or NHEJ pathway to repair their DNA. In order to

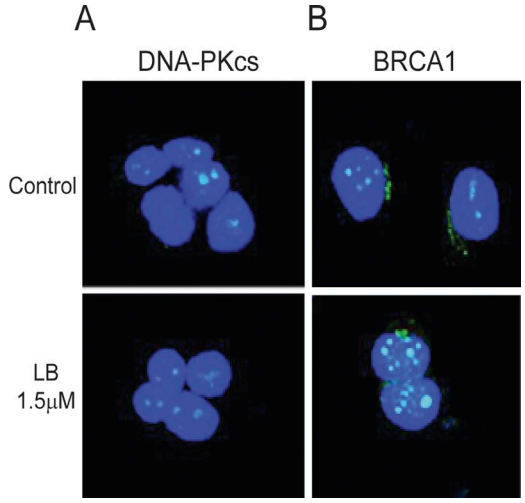

Figure 6. DSB by actin disruption was repaired through HR pathway in MCF-7 cells. MCF-7 cells treated without and with 1.5 μM for 24 h were immunostained with DNA-PKcs (A) and BRCA1 (B) primary antibody followed by secondary antibody conjugated with FITC. Samples were photographed using confocal microscopy at ×400.

investigate which pathway is predominant by actin disruption, the expression level of DNA-PKcs for NHEJ and BRCA1 for HR was examined by confocal image analysis. MCF-7 cells were incubated with 1.5 μM LB for 24 h and washed with cold PBS. Upon permeabilizing and fixing treatment, cells were immunostained with primary antobodies against DNA-PKcs and BRCA1 followed by secondary antibody conjugated with FITC. As shown in Fig. 6A and B, treatment of MCF-7 cells with LB activated BRCA1 protein expression to repair DNA damage. The expression of BRCA1 in the nucleus was particularly prominent in the cells permeabilized before fixation, indicating that DSB by actin disruption was repaired through HR pathway. Therefore, the results suggested that LB induces G2 cell cycle arrest to provide time for repairing DSB by HR pathway.

Discussion

The prominent features of cancer cells include their viability in any circumstance and immortality due to endless cell cycle. These abnormal growth properties of cancer cells are associated with modified actin and a large number of actin-related proteins. Since modified actin dynamics are responsible to tumorigenesis, it is important to understand the mechanism of actin dynamics in cancer for developing a novel anticancer drug (20). Induction of apoptosis has been recognized as a good strategy for the identification of anti-cancer drugs. There have been a number of studies targeting actin cytoskeleton involved in apoptosis in eukaryotic cells including cancer cell, although the effect of actin disruption in apoptosis induction in cancer cells is controversial (21-24). One of the important initiation elements in the apoptotic pathway is the activation of caspases followed by cleavage of PARP, the main substrates of caspase. This is in accordance with our observation of apoptotic bodies and PARP cleaved by LB (Fig. 3).

Of all DNA damage types, DSB is particularly dangerous and induce apoptosis. It has been found that actin disrupting

agents induced phosphorylated H2AX against DSB and arrested cell cycle at G2 phase in MCF-7 breast cancer cells (Figs. 4 and 5). Since phosphorylation of H2AX is considered as a specific marker of DSB induction (25), our data indicate that the phosphorylated H2AX is recruited to their impaired DNA by LB at time- and dose-dependently in human cancer cells. It is known that p53 accumulation plays an important role in the repair and cell cycle arrest (26). p53, downstream molecule of ATM, was understood as a major component of the DNA damage response pathway. After the introduction of DNA injuries the level of p53 protein rises, which in turn induces a transient cell cycle arrest or apoptotic cell death (27). It has been assumed that LB-mediated DSB recruited γH2AX around the DSB regions and then stabilized p53 via ATM-dependent pathway. The contribution of p53 to G2 phase cell cycle arrest involves some of its transcriptional targets such as $p21^{WAF1/CIF1}$, GADD45, and 14-3-3s (28). $p21^{WAF1/CIF1}$ is known to be involved in G2 arrest as well as G1 cell cycle arrest. This protein is accumulated in nucleus at the initiating step of mitosis and interacts with cyclin B1 at the G2 phase (29,30). As shown in Fig. 5, $p21^{WAF1/CIF1}$ is not involved in G2 phase arrest by actin disruption. Therefore, it can be anticipated that DSB induced by LB is implicated with GADD45 or 14-3-3s, which is a downstream signals of p53. The novelty of the present findings is that histone H2AX also is phosphorylated in cells by LB at the G2 boundary. Previous studies demonstrated that actin cytoskeleton plays a central role in early mitosis along with microtubules in eukaryotic cells (31-33). Erk1/2, a key player of the cell cycle belonging to one of the MAPK family, regulate the cell cycle and the progress of apoptosis after DNA damage (6,34). *In vitro*, actin disrupted by latrunculin B or cytochalasin D arrests the cell cycle at G2 phase in yeast or human cells (35). It is suggested that the expression of Erk1/2 was activated by DNA damage and involved in regulating actin disruption in order to control the beginning of mitosis (Fig. 5).

To maintain genetic stability, cells have developed precise mechanisms to repair DSB implicated with the cell cycle. It has been previously suggested that the sister chromatid, present in the late S, G2/M phases of the cell cycle, would favor conservative HR during these phases of the cell cycle, which indicates that HR has a role in DSB repair in S/G2 phase (36). Error prone NHEJ pathway is the simplest way to cure DSB triggered by DNA-PKcs forming juxtaposition with the Ku70/80 heterodimer. BRCA1 may bring about functional interaction with BRCA2, which mediates critically RAD51 strand transferase in HR error-free pathway. Although the exact role of DNA-PKcs and BRCA1 are not clear in DSB repair, actin disruption-mediated DSB may be repaired through HR pathway in that the activation of BRCA1 is higher than that of DNA-PKcs in G2 phase arrest. Although BRCA1 is known to be a mammalian regulator of two DSB repair pathway, this protein mainly controls HR repair in G2 phase (37,38). Considering the results of G2 arrest and BRCA1 expression (Figs. 5 and 6), LB mediated-DSB is repaired through HR pathway in cells.

In summary, actin cytoskeleton is involved in apoptosis through DSB and HR repairing system. DSB responses then activated Erk and p53 which mediated DSB repair and G2 cell cycle arrest. This is the first report of DSB, the most detrimental of DNA damage, induced by actin disruption. Based on our findings and studies of other investigators, we propose a model showing the relationship of actin disruption, DNA damage, DNA damage responses, Erk and p53 in the induction of growth inhibition and apoptosis of cancer cells.

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