Antitumor Activity of SK-7041, a Novel Histone Deacetylase Inhibitor, in Human Lung and Breast Cancer Cells

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Abstract. *Background: A class of synthetic histone deacetylase (HDAC) inhibitors, which are hybrids of trichostatin A and MS-275 were previously developed. In this study, the antitumor effects of SK-7041, one of those novel HDAC inhibitors, was evaluated on lung and breast cancer cell lines. Materials and Methods: Human lung and breast cancer cells, as well as normal human bronchial epithelial (NHBE) cells were treated with SK-7041, and results were compared with those of cells treated with suberoylanilide hydroxamic acid (SAHA). Results: SK-7041 induced time-dependent histone hyperacetylation and showed more potent cytotoxicity than SAHA in cancer cells. These antiproliferative effects of SK-7041 were due to apoptotic cell death caused by G2/M-phase arrest and to a lesser extent to G1 arrest. Moreover, SK-7041 inhibited cancer cell proliferation more selectively than NHBE cell proliferation. Conclusion: These results suggest that SK-7041 may have potential anticancer activity.*

In eukaryotic cells, histone acetylation/deacetylation is an important aspect of transcriptional regulation. Hyperacetylated chromatin is associated with transcriptionally active genes, whereas hypoacetylated chromatin is associated with transcriptionally silent genes. The ε-amino groups of lysine residues in the NH2-terminal tails of histones H2A, H2B, H3 and H4 are modified by histone acetyltransferase (HAT) and histone deacetylase (HDAC) (1, 2). HAT catalyzes the addition of acetyl groups to these lysine residues in core histones, whereas HDAC removes these acetyl groups. Recently, the HDAC enzyme family was identified and is composed of three distinct classes: Class I (HDAC 1, 2, 3 and 8) members are homologues of yeast Rpd3 deacetylase; Class II (HDAC 4, 5, 6, 7, 9, 10 and 11) are homologs of yeast Hda1 deacetylase (3); whereas Class III are homologs of yeast Sir2 and require cofactor NAD+ for their deacetylase activity (4). The variety and abundance of HDAC isotypes imply that these proteins have pivotal cellular functions and that they may show substrate specificity. In addition, it is known that the deregulation of HDAC activity can cause malignant disease in humans (5) and a number of HDAC inhibitors have been found to induce tumor cells to undergo growth arrest, differentiation, or apoptotic cell death (5, 6).

Structurally, the HDAC inhibitors are composed of a functional moiety, a cap structure and a linker, which connects the cap and functional moiety. Crystallographic studies using trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), have shown that the hydroxamic acid functional moiety interacts with the catalytic site of HDACs, and, thus, causes HDAC inhibition (7). TSA, a *Streptomyces* product, contains hydroxamic acid as a functional moiety and has potent inhibitory effects on HDACs at nanomolar concentrations, but it has the drawbacks of instability and low bioavailability (8, 9). MS-275, a synthetic benzamide derivative, has a pyridyl ring cap structure and better physicochemical properties than TSA (10, 11), but it has relatively low potency, *i.e.*, HDAC inhibition at micromolar concentrations.

To maintain the potency and enhance physicochemical properties of TSA, its cap structure was replaced with the pyridyl ring of MS-275. Further incorporation of a

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Key Words: SK-7041, histone deacetylase inhibitor, lung cancer, breast cancer, apoptosis.

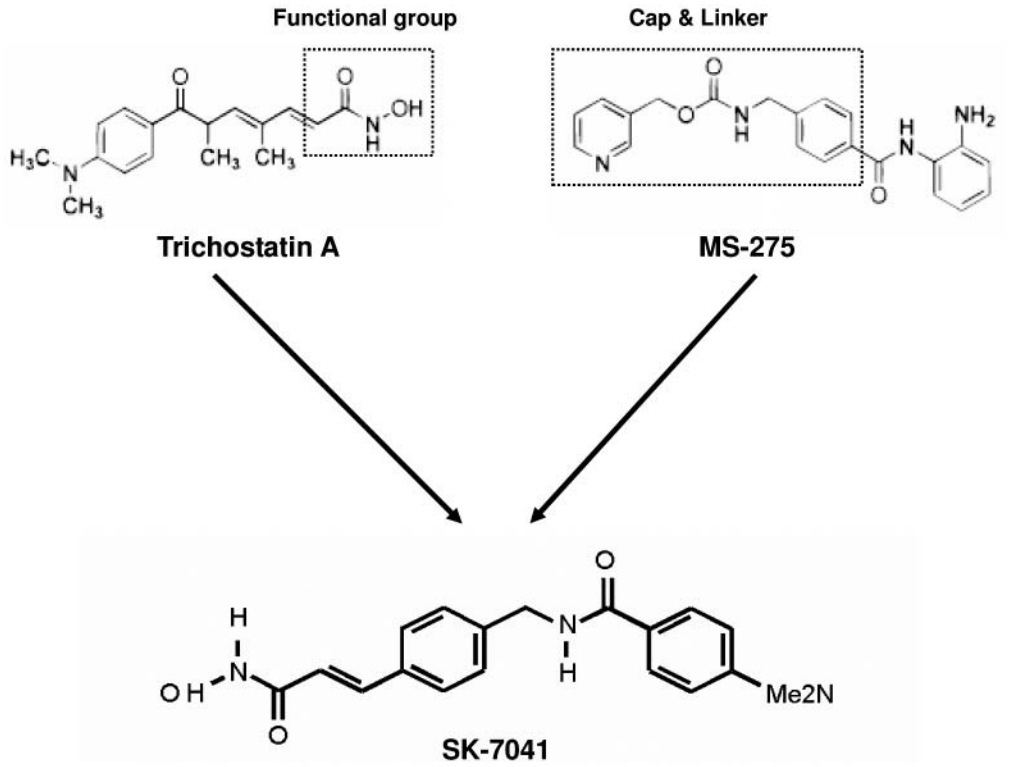

Figure 1. *Molecular design of SK-7041.*

1,4-phenylene carboxamide linker and modification of the 4-(pyrrolidin-l-yl)phenyl group of the cap ring then generated the set of novel hydroxamic acid-based HDAC inhibitors (Figure 1) (12). In a previous study, we measured the inhibitory effects of several novel synthetic HDAC inhibitors on purified HDACs from SNU-16, a gastric cancer cell line. Of those examined, SK-7041 most potently inhibited HDAC in a concentration-dependent manner (13). Moreover, HDAC inhibition was achieved at nanomolar concentrations (IC_{50}, 172 nM) of SK-7041, which was similar to that by Trichostatin A (IC_{50}, 53 nM). MS-275 inhibited HDAC activity at the micromolar level (IC_{50}, 2195 nM) and another novel HDAC inhibitor, SK-7068, also inhibited HDAC activity at the nanomolar level (IC_{50}, 205 nM). In addition, both SK-7041 and SK-7068 inhibited HDAC 1 and HDAC 2 more than the other HDAC isotypes (13). Given these previous results, the effect of SK-7041 on histone acetylation, cell proliferation, the cell cycle, and apoptosis was investigated in human lung and breast cancer cell lines. The SK-7041 results were compared with those of SK-7068 and another promising HDAC inhibitor, SAHA.

Materials and Methods

Chemicals. SK-7041, SK-7068 and suberoylanilide hydroxamic acid (SAHA) were provided by In2Gen (Seoul). For experiments, all the drugs were prepared in 10 mM stock solutions in dimethyl sulfoxide (DMSO, Sigma Chemical Co., St. Louis, MO, USA) and stored in small aliquots at –20˚C.

Cell culture. Human lung cancer cell lines (A549, NCI-H23, and NCI-H1299) and breast cancer cell lines (MDA-MB-231, MCF-7, and SK-BR-3) were obtained from the Korean Cell Line Bank. Each cell line was grown in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum and gentamycin (10 μg/ml). Normal human bronchial epithelial (NHBE) cells were obtained from Clonetics (Walkersville, MD, USA), and grown in bronchial epithelial growth medium (Clonetics). All cells were incubated under standard culture condition (20% O_2 and 5% CO_2; 37˚C).

Cell growth inhibition assays. Tetrazolium-dye (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide [MTT]) (Sigma) assays were used to evaluate the growth inhibitory effects of SK-7041, SK-7068 and SAHA, as previously described (14). Predetermined numbers of cells were seeded in 96-well plates and incubated for 24 h at 37˚C. Aliquots of diluted drugs were added to the wells and cultured at 37˚C for 72 h. Fifty μl of MTT solution (2 mg/ml) were added to each well and incubated for 4 h at 37˚C. Media were then removed and formazan crystals were dissolved by adding 200 μl of DMSO solution/well. The plates were shaken for 30 min at room temperature. Cell viabilities were determined by measuring absorbance at 550 nm in a microplate reader (Spectra Classic, Tecan Co., Austria). Experiments were performed in groups of six at each drug concentration and were repeated three times.

Acid-soluble nuclear protein preparation. For histone preparation, cultured cells were harvested and washed with phosphate buffered saline (PBS). Cells were then pelleted and resuspended in 1 ml ice-cold lysis buffer (10 mM Tris•HCl [pH 6.5], 50 mM sodium bisulfite, 1% Triton X-100, 10 mM $MgCl_2$, and 8.6% sucrose). The suspension was homonized by 2 strokes with Dounce homogenizer. Nuclei were pelleted by centrifugation at 1,200 rpm for 5 min and washed 3 times with 1 ml of lysis buffer. Pellets were treated with 1 ml of Tris•EDTA solution (10 mM Tris·HCl [pH 7.4] and 13 mM EDTA), pelleted nuclei were resuspended in ice-cold water and sulfuric acid was then added to a final concentration of 0.2 M. Samples were then vortexed, incubated on ice for 1 h and centrifuged at 13,000 rpm for 10 min at 4˚C. Proteins obtained in the supernatant were precipitated with 1 ml of acetone overnight at –20˚C. Precipitated proteins were collected by centrifugation at 13,000 rpm for 10 minutes at 4˚C, air dried, and resuspended in 50 μl of water.(13)

Immunoblot analysis of isolated histones. Histones (10 μg), isolated by acid extraction as described above, were then separated by 15% SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. Hyperacetylated histones were detected using antibodies specifically recognizing the hyperacetylated forms of histone H3 and H4 (Upstate Biotechnology, Charlottesville, VA, USA). Rabbit antibodies (Upstate Biotechnology) were used as secondary antibodies. To control for protein loading, gels worked up in parallel were stained with Coomassie blue.

Cell cycle analysis. Cells were harvested by trypsinization and cell suspensions were washed twice in PBS, fixed in 70% ethanol and stored at –20˚C until required for analysis. Before analysis, cell suspensions were washed with PBS and digested with RNase A (50 μg/ml) for 15 min at 37˚C and then stained with propidium iodide (PI) solution (50 μg/ml). Cell DNA contents (10,000 cells/experimental group) were determined using a FACSCalibur flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA) equipped with a ModFit LT program (Verity Software House Inc.).

Annexin V/PI assay for apoptosis. Fluorescein-conjugated Annexin V (Annexin V-FITC) apoptosis detection kits (BD PharMingen, San Diego, CA, USA) were used to quantitatively determine percentages of apoptotic cells. In brief, cells were harvested and cell suspensions were washed twice in PBS, and resuspended in 1 x binding buffer at $1x10^6$ cells/ml; 100 μl of these solutions were then transferred ($1x10^5$ cells) to a 5 ml tube. Annexin-V (5 μl) and PI (10 μl, 50 μg/ml) were then added and incubated for 15 min at room temperature in the dark. 1 x binding buffer (400 μl) was then added to each tube and apoptotic cell counts were determined by flow cytometry within one hour.

Chromosomal DNA isolation and ladder formation assays. For DNA preparation, cells were harvested by centrifugation and then suspended in 500 μl lysis buffer (50 mM Tris-Cl [pH 8.0], 10 mM EDTA and 0.5% laurylsarcosine) containing 50 μg/ml of RNase A. After incubation at 37˚C for 1 h, proteinase K was added to cell lysates at a concentration of 50 μg/ml and samples were further incubated at 55˚C for 4 h. Chromosomal DNA was purified by phenol/chloroform extraction and precipitated with 100% ethanol and 3 M sodium acetate. Precipitated DNA was washed twice with 70% ethanol and quantified spectrophotometrically. Purified DNA (20 μg) was electrophoresed in 1.5% agarose gel and visualized under UV light after standard ethidium bromide staining.

Results

SK-7041 induced histone acetylation in human lung and breast cancer cell lines. Immunoblotting was used to determine whether SK-7041 induced core histone acetylation in cultured human lung and breast cancer cells. The A549 and MDA-MB-231 cells were treated with 1 μM of SK-7041 for 3, 6 and 9 h and acetylated histone H3 and H4 levels were determined. As shown in Figure 2, A549 and MDA-MB-231 showed low basal levels of H3 and H4 acetylation. However, treatment with SK-7041 induced core histone H3 and H4 acetylation within 3 h in both cell lines.

SK-7041 had an antiproliferative effect on human lung and breast cancer cell lines. The cytotoxicities of SK-7041, SK-7068, and SAHA were assayed. The IC_{50} values [drug concentration (μM) at which cell growth is inhibited by 50%] of these agents were determined by MTT assay in each cell line (Table I; Figures 3 and 4). SK-7041 was found to have potent antitumor activity in all cancer cell lines and was more potent than either SK-7068 or SAHA (Table I). The IC_{50} values of SK-7041 in the cell lines tested ranged from 0.26 to 1.01 μM. MCF-7 cells are estrogen receptor (ER)-positive, whereas MDA-MB-231 and SK-BR-3 cells are ER-negative. All three of these breast cancer cells proved to be sensitive to SK-7041 regardless of ER positivity.

SK-7041 treatment induced cell cycle arrest in human lung and breast cancer cell lines. The cell cycle profiles of A549 and MDA-MB-231 cell lines were analyzed after treatment with SK-7041 (1 μM; 6, 12, 18, 24 and 36 h) (Figures 5 and 6). After treatment with SK-7041, the percentage of cells in G2/M-phase increased in 6 hours and this increased with time. The sub-G1 population increased after 12 ~ 18 h of treatment. These observations suggest that apoptotic cell death is caused by significant G2/M-phase arrest in both cell lines. Additionally, the percentage of cells at G1 increased after 12 h of treatment in A549 (Figure 5C) and was maintained within similar ranges for 36 h in MDA-MB-231 (Figure 6C). For the 36 h, a reduced percentage of cancer cells at S phase was maintained persistently in both cell lines. These observations suggest that G1 arrest occurred gradually with the passage of time. Another HDAC inhibitor, SK-7068, produced similar results (Figures 5D and 6D).

SK-7041 induced apoptosis in human lung and breast cancer cell lines. The population of sub-G1 cells increased after 12 ~ 18 h of treatment with SK-7041 in A549 and MDA-MB-231 cells, implying that SK-7041 induced apoptotic cell death in these

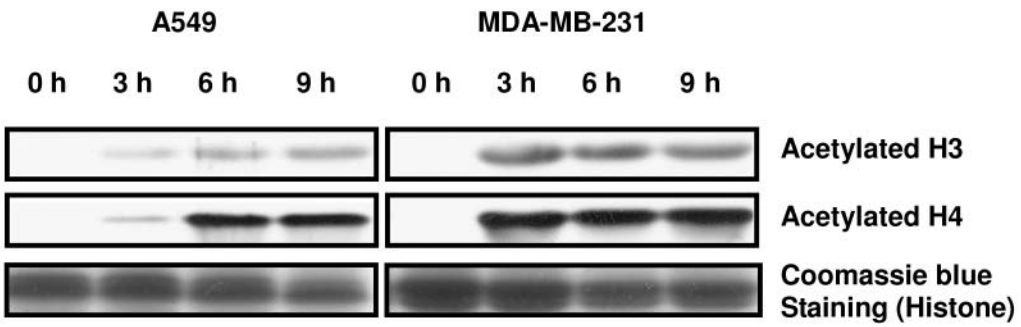

Figure 2. *Induction of histone H3 and H4 acetylation by SK-7041 in A549 and MDA-MB-231 cells. Cells were treated with SK-7041 (1 μM) for the indicated times and histone acetylation was detected by immunoblotting. Histones were stained with Coomassie blue to determine blot loadings.*

cell lines. SK-7041-induced apoptotic cell death was confirmed using Annexin V/PI assays, which detect the externalization of phosphatidylserine during apoptosis. Annexin V/PI stainings were performed on cancer cells treated with SK-7041 for 24 and 48 h, respectively. As shown in Figure 7, the percentage of apoptotic cancer cells after SK-7041 treatment increased with time. SK-7041-induced apoptotic cell death was also confirmed by using chromosomal DNA ladder assays. As shown in Figure 8, chromosomal DNA fragmentation in cancer cells treated with SK-7041 increased with time.

Cancer cells were more sensitive to SK-7041-mediated cell killing than normal cells. To determine whether cancer cells were more sensitive to SK-7041 than normal cells, the effect of SK-7041 was investigated on lung cancer cell lines (A549, NCI-H23 and NCI-H1299) and NHBE cells. Cell growth inhibition assays showed that the lung cancer cell lines were more sensitive to SK-7041-mediated cytotoxicity than the NHBE cells (Figure 9). The IC_{50} values of SK-7041 in A549, NCI-H23, and NCI-H1299 were 0.79, 0.70 and 0.98 μM, respectively (Table I). On the other hand, the IC_{50} of SK-7041 in NHBE cells was 2.73 (±0.08) μM, *i.e.*, up to four times higher than those of lung cancer cell lines. The abilities of SK-7041 and SAHA to selectively inhibit cancer cell growth were compared. As shown Table I, the IC_{50} values of SAHA in A549, NCI-H23, and NCI-H1299 were 3.87, 3.45, and 5.23 μM, respectively, whereas the IC_{50} of SAHA in NHBE cells was 12.65 (±0.65) μM, *i.e.*, up to four-times higher than those of lung cancer cell lines. Ratios of IC_{50} values in NHBE cells and cancer cell lines [IC_{50} (NHBE cell) / IC_{50} (cancer cell)] were similar for SK-7041 and SAHA, suggesting that SK-7041 and SAHA have similar cancer cell-selective cytotoxicities.

Table I. *The IC_{50} values of the different drugs in the various cell lines.*

	SK-7041	SK-7068	SAHA
Lung cancer cell lines			
A549	0.79±0.06 μM	0.93±0.05 μM	3.87±0.31 μM
NCI-H23	0.70±0.03 μM	0.77±0.04 μM	3.45±0.19 μM
NCI-H1299	0.98±0.09 μM	1.21±0.21 μM	5.23±0.25 μM
Breast cancer cell lines			
MDA-MB-231	1.01±0.28 μM	1.33±0.25 μM	5.70±0.35 μM
MCF-7	0.72±0.09 μM	1.37±0.20 μM	2.70±0.36 μM
SK-BR-3	0.26±0.02 μM	0.32±0.02 μM	1.99±0.12 μM

The effects of SK-7041, SK-7068, and SAHA on the proliferations of human lung and breast cancer cell lines were analyzed using MTT assays (Figures 3 and 4). The IC_{50} values shown represent means±SD. Experiments were performed in sets of 6 replicate wells at each drug concentration and independently in triplicate.

Discussion

Worldwide, lung and breast cancer predominate among new cancer cases and are responsible for most cancer deaths. In Korea, lung cancer is the second most common cancer in men and breast cancer is the first most common in women (15). Despite recent advances in therapy, the majority of patients require systemic chemotherapy and more effective, less toxic approaches are needed.

Recently, epigenetic gene inactivation has emerged as an important tumorigenic step. Many target genes, such as, tumor suppressor genes, cell cycle-associated genes, differentiation genes and DNA repair genes are regulated by epigenetic transcription mechanisms. Moreover, histone acetylation/deacetylation is one of the most important epigenetic processes identified in cancer and thus, the targeting of epigenetic transcriptional regulation might provide an effective approach to cancer treatment. To date, several structural classes of HDAC inhibitors have been identified: i) short-chain fatty acids (*e.g.,* butyrates), ii) hydroxamic acids (*e.g.,* TSA, SAHA, oxamflatin and cyclic hydroxamic acid-containing peptide [CHAP] compounds), iii) cyclic tetrapeptides containing a 2-amino-8-oxo-9,10-epoxy-decanoyl moiety (*e.g.,* trapoxin), iv) cyclic peptides not containing the 2-amino-8-oxo-9,10-epoxy-decanoyl

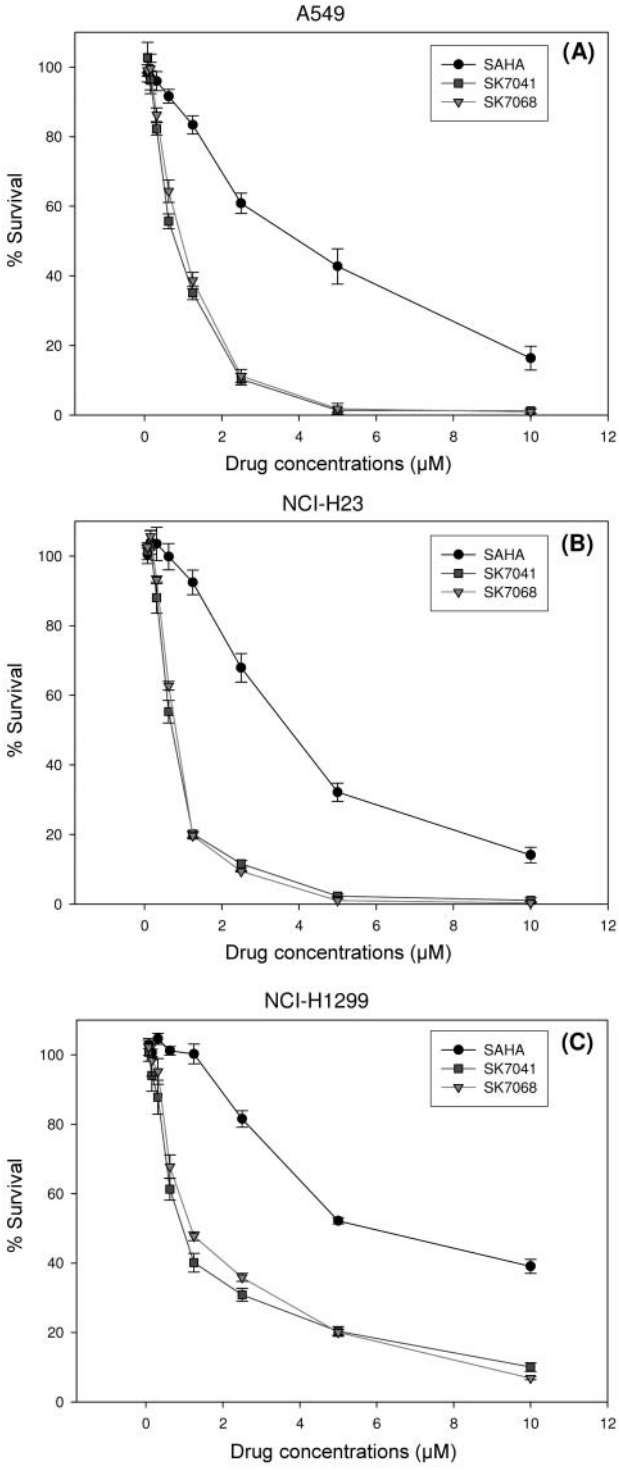

Figure 3. *Antiproliferative effects of SK-7041, SK-7068 or SAHA in the human lung cancer cell lines (A) A549, (B) NCI-H23 and (C) NCI-H1299. The effects of SK-7041, SK-7068, or SAHA on the proliferations of human lung cancer cell lines were analyzed. Cells were seeded into 96-well culture plates and treated with each drug for 3 days and then treated with 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) for 4 h. Cell viability was determined by measuring absorbances.*

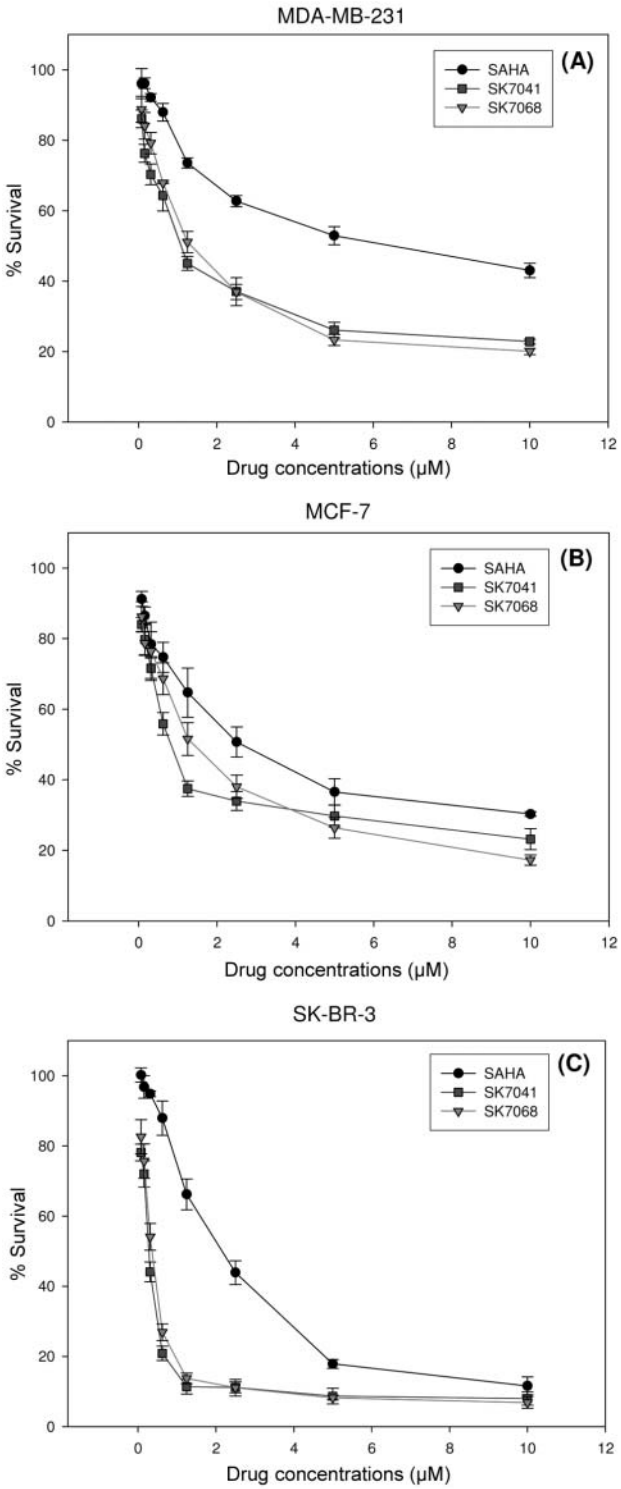

Figure 4. *Antiproliferative effects of SK-7041, SK-7068 or SAHA in human breast cancer cell lines (A) MDA-MB-231, (B) MCF-7 and (C) SK-BR-3. The effects of SK-7041, SK-7068, and SAHA on the proliferations of human breast cancer cell lines were analyzed. Cells were seeded into 96-well culture plates and treated with each drug for 3 days and then treated with 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) for 4 h. Cell viability was determined by measuring absorbances.*

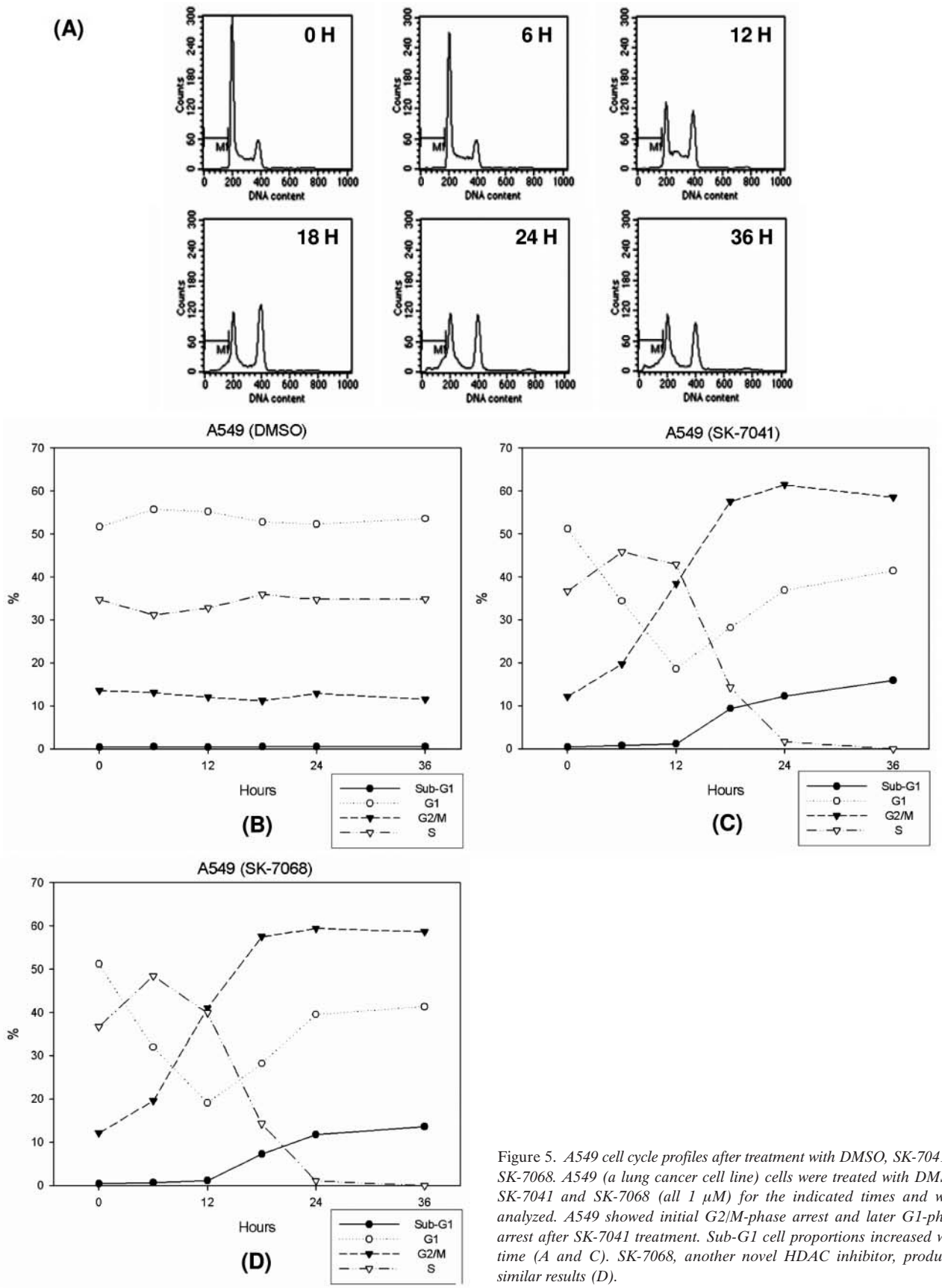

Figure 5. *A549 cell cycle profiles after treatment with DMSO, SK-7041 or SK-7068. A549 (a lung cancer cell line) cells were treated with DMSO, SK-7041 and SK-7068 (all 1 μM) for the indicated times and were analyzed. A549 showed initial G2/M-phase arrest and later G1-phase arrest after SK-7041 treatment. Sub-G1 cell proportions increased with time (A and C). SK-7068, another novel HDAC inhibitor, produced similar results (D).*

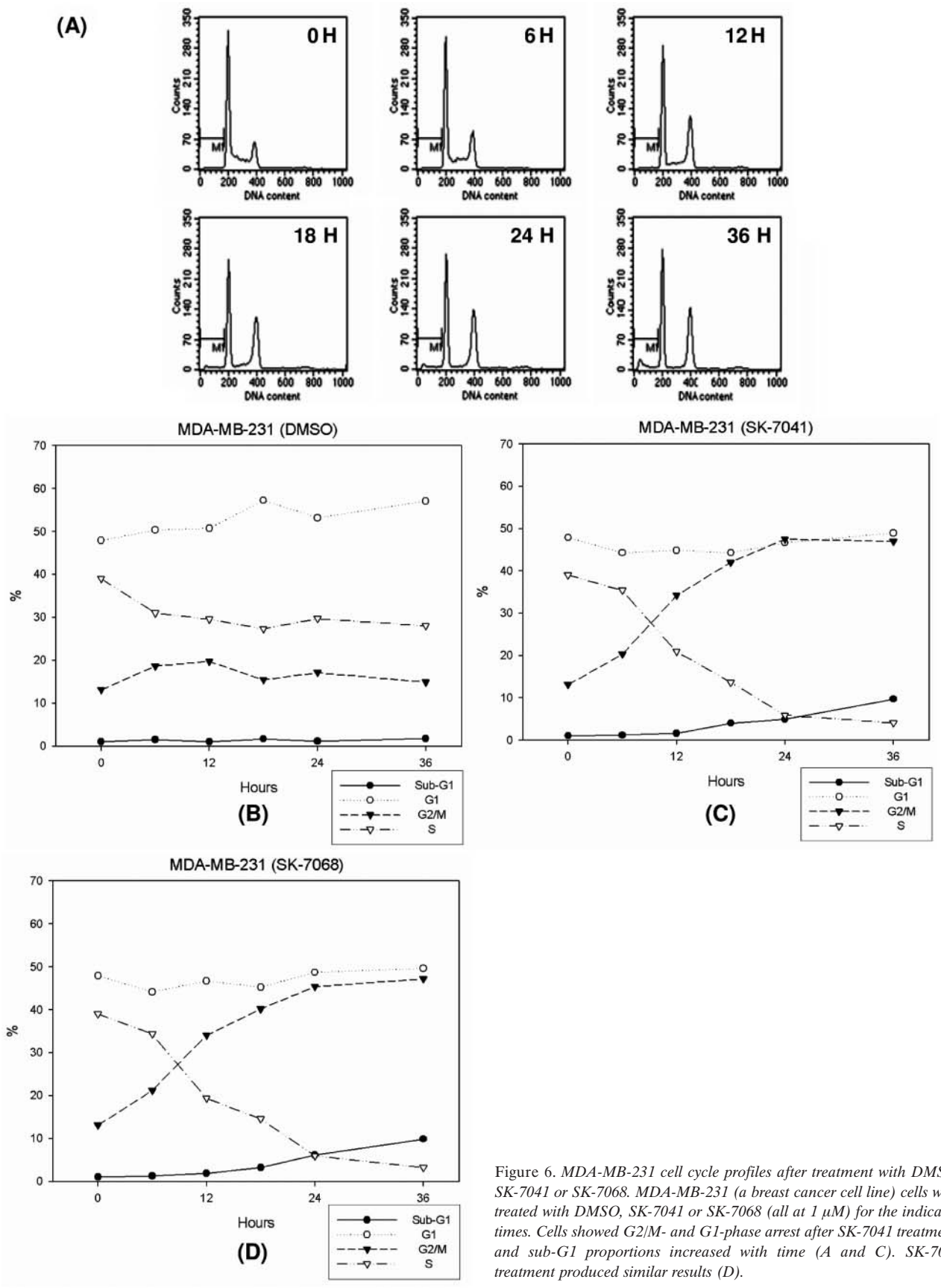

Figure 6. *MDA-MB-231 cell cycle profiles after treatment with DMSO, SK-7041 or SK-7068. MDA-MB-231 (a breast cancer cell line) cells were treated with DMSO, SK-7041 or SK-7068 (all at 1 μM) for the indicated times. Cells showed G2/M- and G1-phase arrest after SK-7041 treatment, and sub-G1 proportions increased with time (A and C). SK-7068 treatment produced similar results (D).*

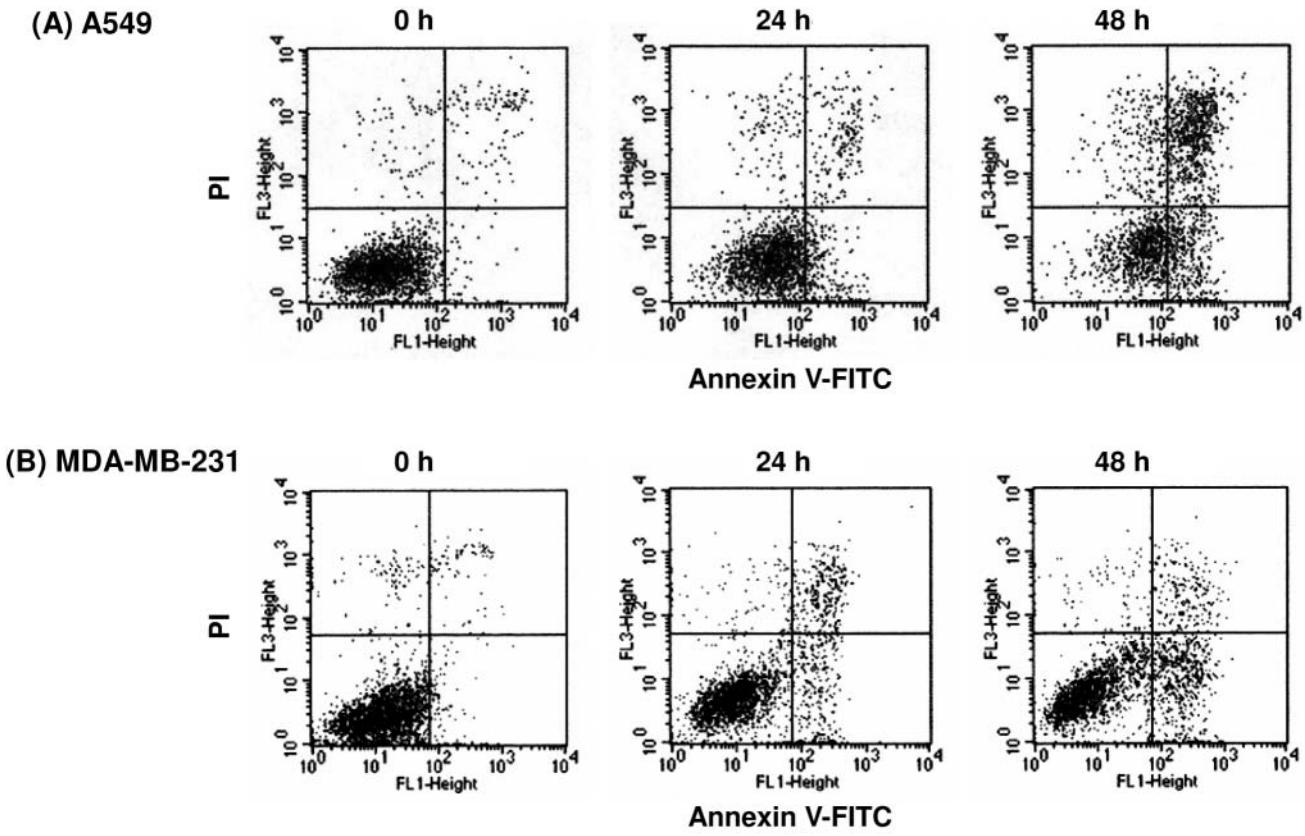

Figure 7. *The externalization of phosphatidylserine during SK-7041-induced apoptosis. Cells treated with SK-7041 (1 μM) for 24 or 48 h were stained with annexin V/PI, and subjected to flow cytometry. Proportions of cells in the right lower quadrant dramatically increased with time, indicating that SK-7041 increased phosphatidylserine externalization and apoptosis.*

moiety (*e.g.,* FR228 and apicidin) and v) benzamides (*e.g.,* MS-275). Of these HDAC inhibitors, several are in clinical trials as cancer therapeutics and to date SAHA is one of the most promising drugs (2, 6, 16-18).

SK-7041, a novel hydroxamic acid-based HDAC inhibitor, was designed for maintenance of potency and improved physicochemical properties. To achieve this, the cap and linker structure of trichostatin A was replaced with that of MS-275 (Figure 1). TSA has a dimethylamino-phenyl cap group and causes the nonselective inhibition of class I and II HDACs. The hybrid synthetic HDAC inhibitor, cyclic hydroxamic acid-containing peptide (CHAP), was constructed from TSA and trapoxin; it has a hydroxamic acid functional moiety and a modified cyclic tetrapeptide cap structure. CHAP shows substrate specificity for HDAC 1 over HDAC 6 (19). We hypothesized that chemical modulation of the cap structure may enhance the enzyme selectivities of HDAC inhibitors. SK-7041 was found to target HDAC 1 and HDAC 2 compared with other HDAC subtypes (13). The presence of large numbers of HDAC isotypes suggests that the isotypes have individual substrate specificities and functions. Thus, the development of isotype-specific HDAC inhibitors might lead to the specific regulation of gene transcriptions that facilitate the tumorigenic progress. This hypothesis requires further consideration, especially from the point of view of modifying cap structures to produce more specific HDAC inhibitors. In addition, we believe that such structural modifications may enhance the stability and bioavailability of SK-7041 and the finding that SK-7041 significantly reduced the growth of implanted tumors *in vivo* supports this idea (13).

In the present study, SK-7041, like the other HDAC inhibitors, also effectively inhibited cancer cell proliferation and this inhibition was achieved in all lung and breast cancer cell lines studied. In addition, SK-7041 was found to be more cytotoxic than SK-7068, another newly developed HDAC inhibitor, and than SAHA. Cancer cell lines were found also to be more sensitive to SK-7041-mediated growth inhibition than NHBE cells and the ratios of the IC_{50} values of SK-7041 and SAHA in NHBE cells and cancer cell lines were similar. These results suggest that SK-7041 is more potent than SAHA and might have a comparable cancer cell-specific cytotoxicity. In addition, SK-7041 effectively

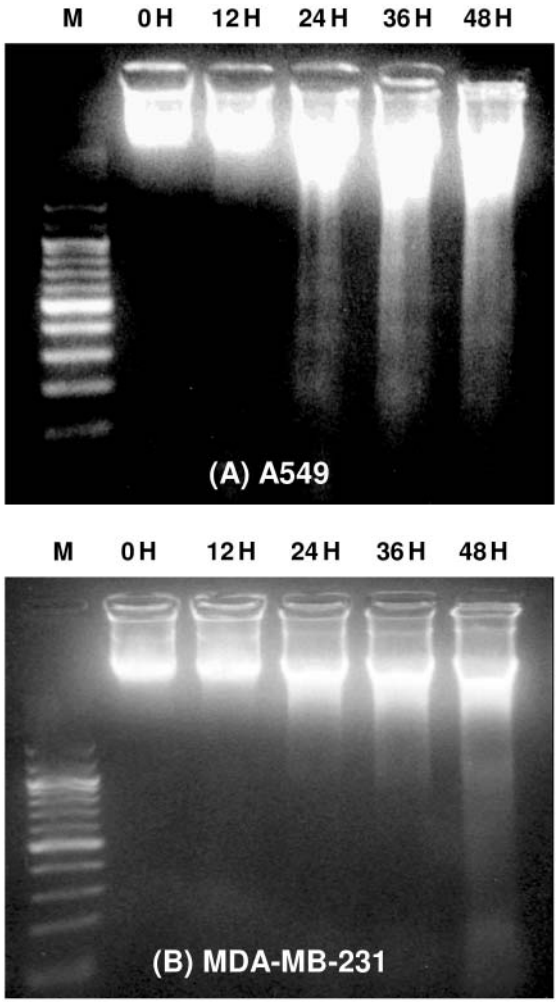

Figure 8. *The induction of DNA fragmentation by SK-7041. Chromosomal DNA was extracted from cancer cells treated with SK-7041 (1 μM) for the indicated times and electrophoresed in 1.5% agarose gels. DNA size marker (M) was used as a running control. SK-7041 increased apoptosis-induced DNA fragmentation with time, in both lung and breast cancer cell lines.*

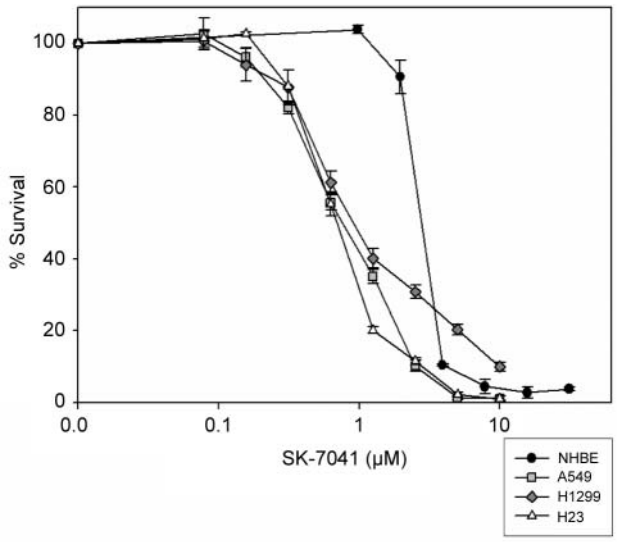

Figure 9. *Lung cancer cells were more sensitive to SK-7041 than normal human bronchial epithelial (NHBE) cells.*

repressed tumor xenograft growth in a human lung cancer nude mouse model (A549) (13).

It has been reported that SAHA induces G1 cell cycle arrest and/or G2/M cell cycle arrest in a cell-type-, dose- and time-dependent manner (20-22). We found that G2/M-phase arrest predominated after SK-7041 treatment in the lung and breast cancer cell lines and our findings support the notion of G1-phase arrest. In addition, apoptotic cell death by SK-7041 was found to increase with time after SK-7041 treatment. Park *et al.* demonstrated that the antiproliferative effects of SK-7041 and SK-7068 on a human gastric cancer cell line (SNU-16) mainly involved aberrant mitosis and apoptosis. When these researchers treated the SNU-16 gastric cancer cell line with SK-7041 or SK-7068, cell cycle analyses showed that the growth-inhibitory effects of these drugs were mainly associated with accumulations of cancer cells in the G2/M-phase and apoptosis and in part with later G1 arrest. In that study, a lack of G2 cyclin/cdk inactivation was observed and the authors suggested that cells sensitive to SK-7041 or SK-7068 accumulated in the mitotic phase and are committed to the apoptotic process by a G2 checkpoint-independent mechanism (13). Ryu *et al.* also showed that SK-7041 induced apoptosis and cell cycle arrest mainly in the G2/M-phase in pancreatic cancer cell lines (Panc-1 and ASCP-1) (23). Our data for lung and breast cancer cell lines concur with these reports.

Until recently, the anticancer effects of SK-7041 on lung and breast cancer cells have been tested only in two cell lines (A549 and SK-BR-3) (12, 13). However, previous reports only evaluated the antiproliferative activity of SK-7041 using the MTT assay and no further experiments were performed. This study is the first study to evaluate anticancer effects of SK-7041 on various human lung and breast cancer cells systematically. Furthermore, we showed that human cancer cells were more sensitive to SK-7041-mediated cell killing than normal human cells (NHBE cells). In the previous report, selective cytotoxicities of SK-7041 to cancer cells were demonstrated using nontransformed rat intestinal epithelial cells (13).

In summary, SK-7041, a novel synthetic HDAC inhibitor, showed potent cytotoxicity in lung and breast cancer cell lines. These inhibitory effects on cancer cell proliferation resulted from the apoptotic cell death caused by G2/M-phase arrest and, to some extent, G1-phase arrest. In

addition, SK-7041 more selectively inhibited lung cancer cell lines than normal human bronchial epithelial cell. These results demonstrate that SK-7041 is a promising HDAC inhibitor and demonstrate the need for further study.

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