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Downregulation of Akt1 Inhibits Anchorage-Independent Cell Growth and Induces Apoptosis in Cancer Cells

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Abstract

The serine/threonine kinases, Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ, play a critical role in preventing cancer cells from undergoing apoptosis. However, the function of individual Akt isoforms in the tumorigenicity of cancer cells is still not well defined. In the current study, we used an Akt1 antisense oligonucleotide (AS) to specifically downregulate Akt1 protein in both cancer and normal cells. Our data indicate that Akt1 AS treatment inhibits the ability of MiaPaCa-2, H460, HCT-15, and HT1080 cells to grow in soft agar. The treatment also induces apoptosis in these cancer cells as demonstrated by FACS analysis and a caspase activity assay. Conversely, Akt1 AS treatment has little effect on the cell growth and survival of normal human cells including normal human fibroblast (NHF), fibroblast from muscle (FBM), and mammary gland epithelial 184B5 cells. In addition, Akt1 AS specifically sensitizes cancer cells to typical chemotherapeutic agents. Thus, Akt1 is indispensable for maintaining the tumorigenicity of cancer cells. Inhibition of Akt1 may provide a powerful sensitization agent for chemotherapy specifically in cancer cells.
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Keywords: Akt1, apoptosis, antisense, oligonucleotide, cancer, combination treatment.

Introduction

For an animal to maintain tissue homeostasis, it must keep the balance between cell growth and cell death [1,2]. Perturbation of the balance, either too much cell growth or too little cell death, will result in tumorigenesis [1,3]. Recently, Akt has been implicated to play a critical role in preventing cancer cells from undergoing apoptosis [4,5]. Akt is a serine/threonine protein kinase originally identified as a cellular homologue of viral oncogene Akt8 [6]. It was also cloned as a kinase that is homologous to cAMP dependent kinase (PKA) and protein kinase C (PKC) [7,8]. The three isoforms of Akt (Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ) share high homology with 78% to 84% protein sequence identity [4,9]. The domain structures of Akts are well conserved from human to *C. elegans* [4]. They all contain an N-terminal pleckstrin homology (PH) domain, a kinase domain and a C-terminal hydrophobic domain [4].

Akt is activated through the phosphatidylinositol 3-kinase (PI-3K) pathway upon growth factor stimulation [10,11]. The products of PI-3K, especially phosphatidylinositol [3–5] triphosphate (PIP3) and phosphatidylinositol [3,4] bisphosphate (PIP2), can bind to the PH domain of Akt [12,13]. The binding of PIP3 and PIP2 to PH domain of Akt targets the protein to membrane [14], where it can be phosphorylated and activated by PDK1 and ILK [15–18]. These latter enzymes phosphorylate Akt at threonine 308 and serine 473, respectively [17,18], although the phosphorylation of Ser-473 has also been suggested through an autophosphorylation mechanism [19]. Activated Akt phosphorylates specific targets, including Bad, caspase-9, forkhead transcription factors, IkB kinase kinase (IKK) [20–23], thereby promoting cell survival.

Many lines of evidence indicate that Akts are involved in tumorigenesis. Akt is constitutively activated in PTEN negative cancer cells [24,25]. PTEN is a tumor suppressor gene frequently mutated in many advanced tumors [25,26]. PTEN downregulates the activity of Akt by acting as a phosphatase that reverses the effect of PI-3K [24–26]. Akt1 is amplified in gastric adenocarcinomas [27]. Akt2 is overexpressed in 10% to 20% pancreatic and ovarian cancers [28]. Akt3 is overexpressed in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer cells [29].

Although Akts play critical roles in preventing cells from undergoing apoptosis, it is not clear whether all Akts are required for the tumorigenesis of cancer cells, nor is it clear how important individual Akts are in the process. In this report, we used Akt1 antisense oligonucleotide (AS) to study the function of Akt1 in cancer cell growth and survival. We found that Akt1 AS reduced Akt1 protein expression, inhibited the ability of cancer cells to grow in soft agar, induced

Abbreviations: AS, antisense; MS, mismatch control; oligo, oligonucleotide; Lip, lipofectin; NHF, normal human fibroblast; FBM, fibroblast from muscle; Dox, doxorubicin; PDK, 3-phosphoinositide dependent kinase; ILK, integrin-linked kinase; PKB, protein kinase B; PARP, poly-ADP ribose polymerase; PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis (β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol

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apoptosis, and specifically sensitized cancer cells, but not normal cells, to typical chemotherapeutic agents.

Materials and Methods

All chemicals were from Sigma (St. Louis, MO). AlamarBlue was from BioSource International (Camarillo, CA). Protein concentration was determined using BCA method according to the manufacturer's instructions (Pierce, Rockford, IL).

Cell Lines

All tumor cell lines were obtained from American Type Culture Collection (Rockville, MD). Normal human fibroblast (NHF) and fibroblast from muscle (FBM) were obtained from Clonetics (Walkersville, MD). 184B5 cells were from NIH. Cells were cultured in the conditions provided by the suppliers.

Antisense Oligonucleotide Transfection

The 2′-*O*-methoxyethyl (2′-MOE) chimeric phosphorothioate oligonucleotides for Akt1 antisense-Isis#28949 (AS) and the mismatch control-Isis#104566 (MS) were provided by Isis Pharmaceuticals. The first four and last four bases on the oligos are with 2′-MOE modification. The remaining 10 bases on the oligos are regular deoxynucleotides. Cells were transfected with Akt1 AS or MS oligos using lipofectin reagent (Gibco BRL, Gaithersburg, MD) at a ratio of 3 μl lipofectin/ml Opti-MEM (Gibco BRL) per 100 nM oligo. Four hours after transfection, cells were incubated with normal complete media.

Western Blot Analysis

Rabbit anti-Akt1 antibody was from New England BioLabs (Beverly, MA). Sheep anti-Akt2 antibody was from Upstate Technology (Lake Placid, NY). Anti-cytochrome *c* antibody and anti-PARP antibody were from Pharmingen (San Diego, CA). Immunoblot analysis was performed with the horseradish peroxidase-conjugated goat anti-sheep (Akt2), or goat anti-rabbit IgG (Akt1), or sheep anti-mouse IgG (cytochrome *c* and PARP) by using enhanced chemiluminescence (ECL) Western blotting detection reagent (Amersham, Arlington Heights, IL) as described previously [30].

AlamarBlue Cell Proliferation Assay

The alamarBlue assay was conducted according to the manufacturer's instruction. Briefly, cells in 96-well plates were washed with 200 μl PBS, followed by addition of 200 μl of the complete medium containing 10% alamarBlue to each well. After 3 hours at 37°C in a CO_2 incubator, the plate was read on fmax SoftmaxPro (Molecular Devices, Sunnyvale, CA) using the following filter pair: Ex544/Em590.

Flow Cytometry Analysis

Cells were harvested by pooling attached and detached cells and pelleted by centrifugation at 800×*g* for 5 minutes at 4°C. The cells were washed with PBS and resuspended in 0.5 ml ice-cold staining solution (5 μg/ml propidium iodide (PI), 40 U/ml RNase A, 0.5% Triton X-100, in PBS). After 1 hour at 4°C in the dark, the DNA content was analyzed using a Beckton Dickinson ExCalibur flow cytometer (San Jose, CA).

Caspase Activity Assay

Cells with 100 μl culture medium in 96-well plate were lysed in 20 μl of lysis buffer (in mM: 10 Hepes, pH 7.5, 35 KCl, 4 $MgCl_2$, 0.1 EDTA, 0.1 EGTA, 0.2 PMSF, 1.0 DTT, 1×protease inhibitor cocktail tablet; Boehringer Mannheim, Mannheim, Germany) at room temperature for 20 minutes, followed by addition of 80 μl of the caspase reaction buffer containing 48 mM Hepes, pH 7.5, 292 mM sucrose, 0.1% CHAPS (Calbiochem, San Diego, CA), 1 mM Ac-DEVD-AMC (Bachem, King of Prussia, PA). After mixing, the plate was read on Cytofluor series 4000 (Applied Biosystems) using the following setting: excitation=360/40, emission=460/40, gain=38, cycle=1. After 3 hours incubation at 37°C, the plate was read again. The units of fluorescence change per hour (dFU/h) are defined as caspase activity.

Soft Agar Growth Assay

The soft agar growth assay was performed as described [31]. These assays were carried out in 10% fetal calf serum. For the bottom layer of agar, 1 ml of 0.5% agar was placed in each 35-mm-diameter well of six-well plates. Then 2 ml of 0.3% top agar containing 1×10^4 cells was layered on top of the solidified layer of bottom agar. After 2 weeks, the colonies were stained with *p*-iodonitrotetrazolium violet and the number of colonies were scored by counting the colonies using the image analysis program Image-Pro Plus (Media Cybernetics, Silver Spring, MD).

Preparation of cell extracts

Cells from 10-cm Petri dishes were harvested and lysed in 200 μl buffer B (in mM: 20 Hepes, pH 7.5, 10 NaCl, 20 EDTA, 1 EGTA, 5 sodium pyrophosphate, 2 sodium orthovanadate, 10 β-glycerophosphate, and 1% NP-40) on ice for 30 minutes. The samples were centrifuged at 12,000×*g* at 4°C for 10 minutes. The supernatants were used as cell extracts.

Preparation of Cytosolic Fractions from MiaPaCa-2 Cells

The isolation of cytosolic fractions from MiaPaCa-2 cells was carried out as described [32]. Briefly, MiaPaCa-2 cells were harvested and washed with ice-cold PBS and resuspended in five volumes of buffer A (in mM: 20 Hepes, pH 7.5, 10 KCl, 1.5 $MgCl_2$, 1 sodium EDTA, 1 sodium EGTA, 1 DTT, and 0.1 PMSF) containing 250 mM sucrose. The cells were homogenized with 10 strokes of a Teflon homogenizer. The homogenates were centrifuged twice at 750×*g* for 10 minutes at 4°C. The supernatant was further centrifuged at 100,000×*g* for 1 hour at 4°C,

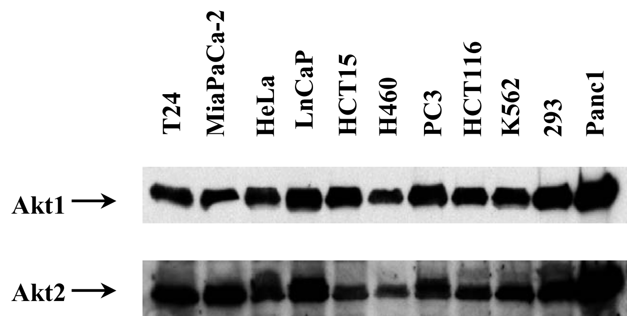

Figure 1. *Akt1 and Akt2 protein levels in cancer cell lines. Cells were cultured and cell extracts were prepared as described in Materials and Methods section. Fifty micrograms of the cell extracts prepared from different cell lines were loaded to 10% SDS polyacrylamide gels and subjected to Western blot analysis using Akt1 and Akt2 antibodies as described in Materials and Methods section.*

and the resulting supernatant was designated as cytosolic fraction.

Results

Protein Level of Akt1 and Akt2 in Different Cancer Cell Lines

We examined the expression levels of Akt1 and Akt2 in a panel of cancer cell lines. Western blot analysis shows that Akt1 and Akt2 proteins are expressed in all the cancer cell lines tested here (Figure 1).

Akt1 AS Reduced Akt1 Protein Level

We used an antisense oligonucleotide (Isis#28949) specific for Akt1 to study the effect of inhibiting Akt1 in cancer cells (Figure 2*A*). A mismatch oligonucleotide (Isis#104566) containing six nucleotide substitutions was used as a control (Figure 2*A*). Twenty-four hours after transient transfection, Akt1 and Akt2 protein levels in these transfected cells were measured by Western-blot analysis. As shown in Figure 2*B*, 250 nM Akt1 AS oligo reduced Akt1 protein level between 47% and 82% in HeLa, MiaPaCa-2, H460, and HCT-15 cells. The Akt2 protein levels, however,

A

Akt1 Antisense (AS): C**T**G**G**C**T**GA**C**AG**A**GT**G**AGG
Akt1 Mismatch (MS) C**GG**A**C**C**G**AT**A**GG**G**TT**T**AGG

B

	HeLa			MiaPaCa-2			H460			HCT15		
	Lip	AS	MS	Lip	AS	MS	Lip	AS	MS	Lip	AS	MS
Akt1 →												
Density →	63,	13,	73,	50,	9,	42	122,	65,	134,	116,	42,	120
% Reduction →		79,	-16,		82,	16		47,	-10,		64,	-3
Akt2 →												
Density →	147,	128,	130,	241,	243,	245 ,	60,	53,	58	146,	165,	148
% Reduction →		13,	12,		-1,	-1,		12,	3.3,		-13,	-1

Figure 2. *Akt1 AS reduced Akt1 protein level in cancer cells. (A) The nucleotide sequences of Akt1 antisense (AS) and mismatch control (MS). (B) HeLa, MiaPaCa-2, H460, and HCT-15 cells were transfected with 250 nM Akt1 AS or MS as described in Materials and Methods section. Twenty-four hours after transfection, cells were harvested and extracts were prepared as described in Materials and Methods. Fifty micrograms of the extracts were subjected to Western blot analysis using Akt1 or Akt2 antibodies as described in Materials and Methods. The density of the protein bands were measured using a Bio-Rad GS-710 calibrated imaging densitometer.*

were not affected by the Akt1 AS oligo (Figure 2*B*). Similar effects were observed in other cancer cell lines including PC3, LnCaP, HT1080, and DLD1 (data not shown).

Akt1 AS Inhibited the Proliferation of Cancer Cells

To test the effect of Akt1 AS on cancer cells, we chose five cancer cell lines from different tissues and performed the alamarBlue cell proliferation assay after treating them with Akt1 AS for various amounts of time (Figure 3). Compared to lipofectin control, Akt1 AS treatment results in a great decrease in the number of viable cells with less than 20% in HT1080, HeLa and MiaPaCa-2 cells forty-eight hour after transfection (Figure 3*A*, *C*, *E*), respectively. Viable cells (25% and 37%) were observed for H460 and HCT15 after 72-hour treatment with Akt1 AS (Figure 3*B* and *D*). The Akt1 MS also showed some nonspecific toxicity in the assay (Figure 3).

Akt1 AS Inhibited the Ability of Cancer Cells to Grow in Soft Agar

One of the hallmarks of cancer cells is their ability to grow in an anchorage-independent manner. So we tested whether Akt1 AS treatment on cancer cells would affect their growth in soft agar. As shown in Figure 4, the number of colonies formed by HT1080 cells after Akt1 AS treatment were only about 20% of that of lipofectin control, whereas the MS oligo–transfected HT1080 cells generated 70% colonies of the lipofectin control (Figure 4). Similar effects were observed for MiaPaCa-2, H460 and HCT15 cells (Figure 4).

Akt1 AS Induced Cancer Cells to Undergo Apoptosis

Akt1 is critical for the survival of cancer cells. According to flow cytometry analysis (Figure 5*A* and *C*), 18.7% of MiaPaCa-2 cells, 24% of HeLa cells, 21% of HT1080 cells, 4% of H460, and 3.5% of HCT-15 cells undergo apoptosis 24 hours after treatment with Akt1 AS oligo, whereas very few cells (e.g., 0.3% for MiaPaCa) were dying in MS oligo–transfected cells. To make sure the cells were dying through apoptosis, instead of through nonspecific necrosis, we examined the status of cytochrome *c* in cytosol and cleavage of poly-ADP ribose polymerase (PARP), two of the biochemical hallmarks of apoptosis. A time course treatment on MiaPaCa-2 cells was carried out using Akt1 AS and MS, and cell extracts as well as cytosolic fractions were prepared from the corresponding cells. Western blot analysis showed that more cytochrome *c* was present in the cytosol of Akt1 AS–transfected cells (Figure 5*B*), indicating the release of

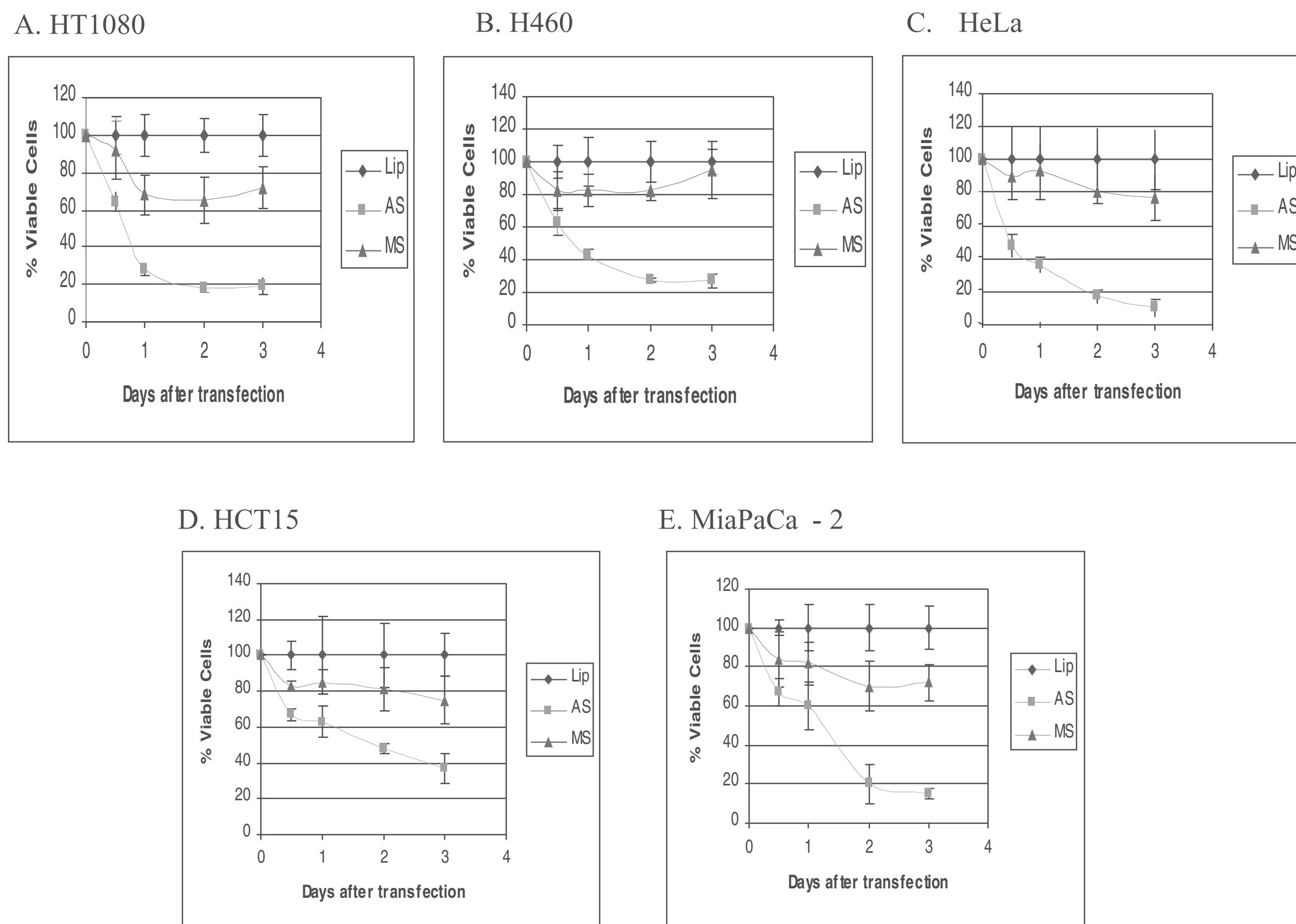

Figure 3. *Akt1 AS inhibits cancer cell proliferation. At day 0, 3000 cells (per well) were plated into 96-well Costar plates. At day 1, cells were transfected with 250 nM Akt1 AS, or MS, or lipofectin control as described in Materials and Methods section. The alamarBlue assay was performed at various time points as described in Materials and Methods. Data are representative of two independent experiments with eight replicates for each point. Shown is the mean ± SD of the eight replicates.*

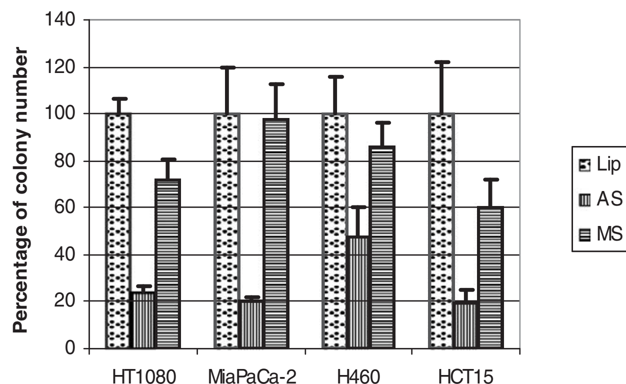

Figure 4. *Akt1 AS oligo inhibited cancer cell growth in soft agar. HT1080 cells, MiaPaCa-2 cells, NCI-H460 cells or HCT-15 cells were transfected with 250 nM Akt1 AS or MS oligos as described in Materials and Methods section. Twenty-four hours after transfection, cells were harvested and 10,000 live cells were plated to a soft agar assay as described in Materials and Methods. Data were expressed as the percentage of colony number with lipofectin transfected cells as 100%. Shown is the mean ± SD of three experiments.*

cytochrome *c* from mitochondria to cytosol. The PARP cleavage started at 20 hours after Akt1 AS transfection, and very little PARP precursor was left at 40 hours after Akt1 AS transfection (Figure 5*B*). The timing of release of cytochrome *c* correlated well with the timing of PARP cleavage (Figure 5*B*), suggesting that cell death occurred through apoptosis instead of nonspecific necrosis.

Akt1 AS Did Not Induce Apoptosis in Normal Cells

We also tested the effect of Akt1 AS treatment on three normal cell lines by FACS analysis. As shown in Figure 6, 24 hours after Akt1 AS treatment, only 0.9% of NHF cells undergo apoptosis whereas Akt1 MS treatment has 0.8% apoptotic cells, although Akt1 AS reduces Akt1 protein in the cells by 75%. A similar effect was observed in other normal cells including FBM and mammary gland epithelial 184B5 cells (Figure 6).

Akt1 AS Sensitized Cancer Cells to Chemotherapeutic Treatment

Because Akt has been shown to protect cancer cells from a variety of apoptotic stimuli-induced cell death [5,33–35], we hypothesized that Akt1 AS would sensitize cancer cells to chemotherapeutic agent-induced apoptosis. We therefore tested the combination effects of Akt1 AS treatment and the

A

AS

Apopt

Counts

relative DNA (peak area)

MS

Apopt 0.3%

Counts

relative DNA (peak area)

B

Control | AS | MS

40 | 24 40 | 24 40 Time (hours)

PARP

Cleaved PARP

Cyto. C

C

Cell line	Apoptotic Population	Akt1 reduction
MiaPaCa-2	18.7%	82%
HeLa	24%	79%
HT1080	21%	65%
H460	4.0%	47%
HCT-15	3.5%	64%

Figure 5. *Akt1 AS induced apoptosis in cancer cells. HeLa, MiaPaCa-2, HT1080, H460 or HCT15 cells were transfected with 250 nM Akt1 AS or MS using lipofectin as described in Materials and Methods section. After 24 hours, FACS analysis was carried out as described in Materials and Methods. (A) FACS analysis for MiaPaCa-2 cells treated with Akt1 AS and MS. (B) MiaPaCa-2 cells were transfected with Akt1 AS or MS at 250 nM concentration for the indicated times. Cells were harvested. Half of them was used to prepare cytosolic fractions, and the other half was used to prepare cell extracts as described in Materials and Methods. 30 μg of cytosolic fractions or 50 μg of cell extracts were subjected to Western blot analysis for cytochrome c and PARP, respectively. (C) Summary of the apoptotic population by FACS analysis and Akt1 protein reduction by Western blot in five cancer cell lines after Akt1 AS treatment.*

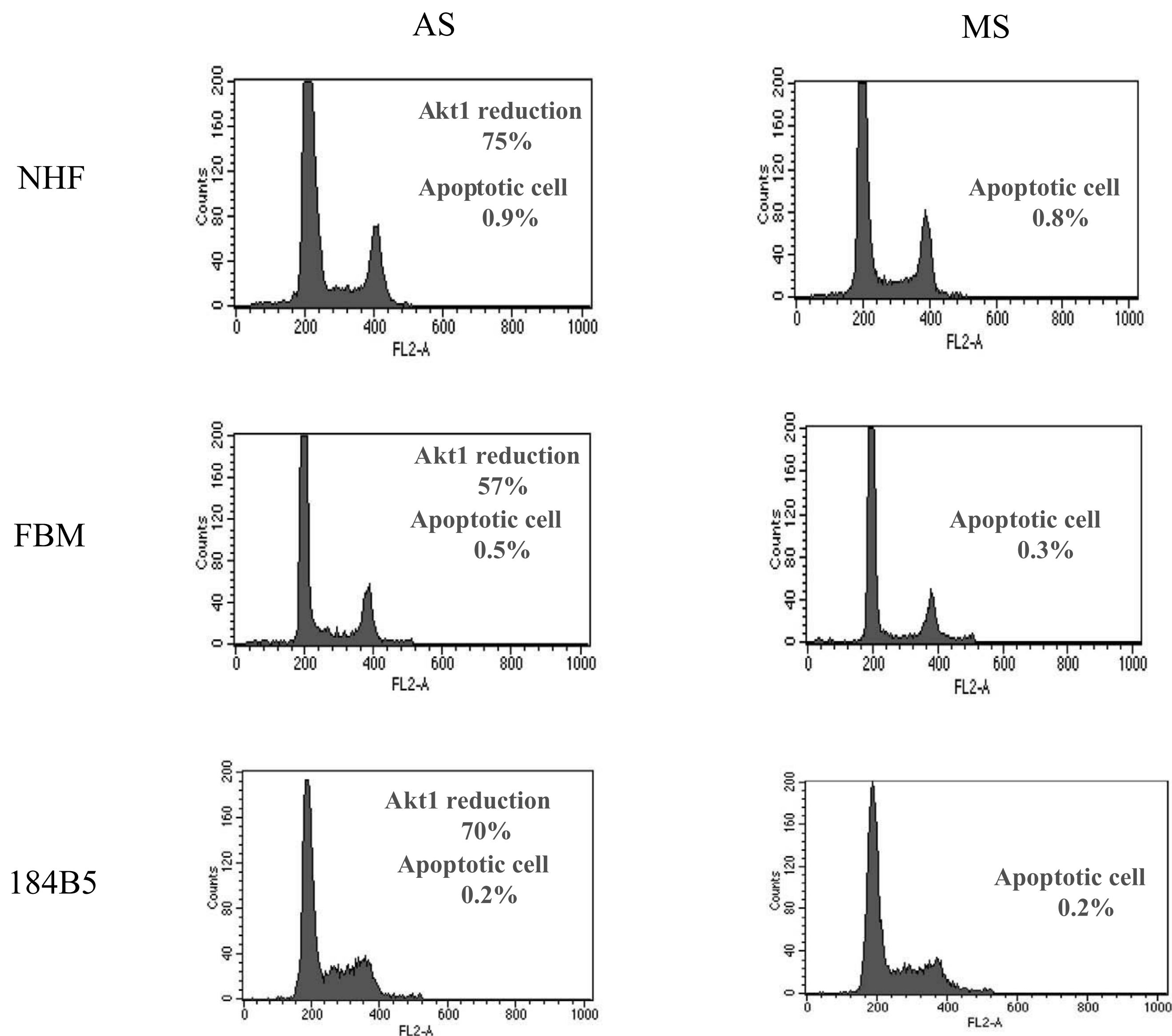

Figure 6. *Akt1 AS had little effect on the survival of normal human cells. NHF, FBM or 184B5 cells were transfected with 250 nM Akt1 AS or MS for 24 hours. Cells were harvested, with one third of the cells subjected to FACS analysis, and the other two-thirds subjected to Western blot analysis for Akt1 as described in Materials and Methods section.*

chemotherapeutic agent etoposide or doxorubicin on H460 and HCT-15 cells, which are quite resistant to etoposide or doxorubicin. As shown in Figure 7*A*, Akt AS treatment alone in H460 cells induced 41 units of caspase activity and doxorubicin treatment alone induced 22 units of caspase activity. However, combination treatment with Akt1 AS and doxorubicin resulted in 92 units of caspase activity, indicating a synergistic effect. On the contrary, the Akt1 MS treatment together with doxorubicin did not result in significant change in caspase activity. The synergistic effect was also observed in HCT15 cells after Akt1 AS and doxorubicin treatment (Figure 7*B*), and in both cell lines with Akt1 AS and etoposide treatment (Figure 7*D* and *E*). In addition to these tumor cell lines, we also tested the effect of Akt1 AS in combination with doxorubicin or etoposide on the NHF cell line. As shown in Figure 7*C* and *F*, either Akt1 AS, or doxorubicin, or etoposide alone generated very little caspase activity, and no synergistic effect was observed.

Discussion

Akt1 Is Critical for Cancer Cell Growth and Survival

Apoptosis is a built-in process that clears away the damaged, unnecessary, or abnormal cells in metazoan organisms [2]. Cancer cells are abnormal cells and differ from normal cells in many aspects [3]. To sustain the tumorigenicity, cancer cells must gain the ability to avoid apoptosis [3]. Akts are key proteins that prevent cancer cells from undergoing apoptosis. It seems that three Akts (Akt1, Akt2, and Akt3) have the same substrate specificity because they all can phosphorylate Bad *in vitro* (Liu X,

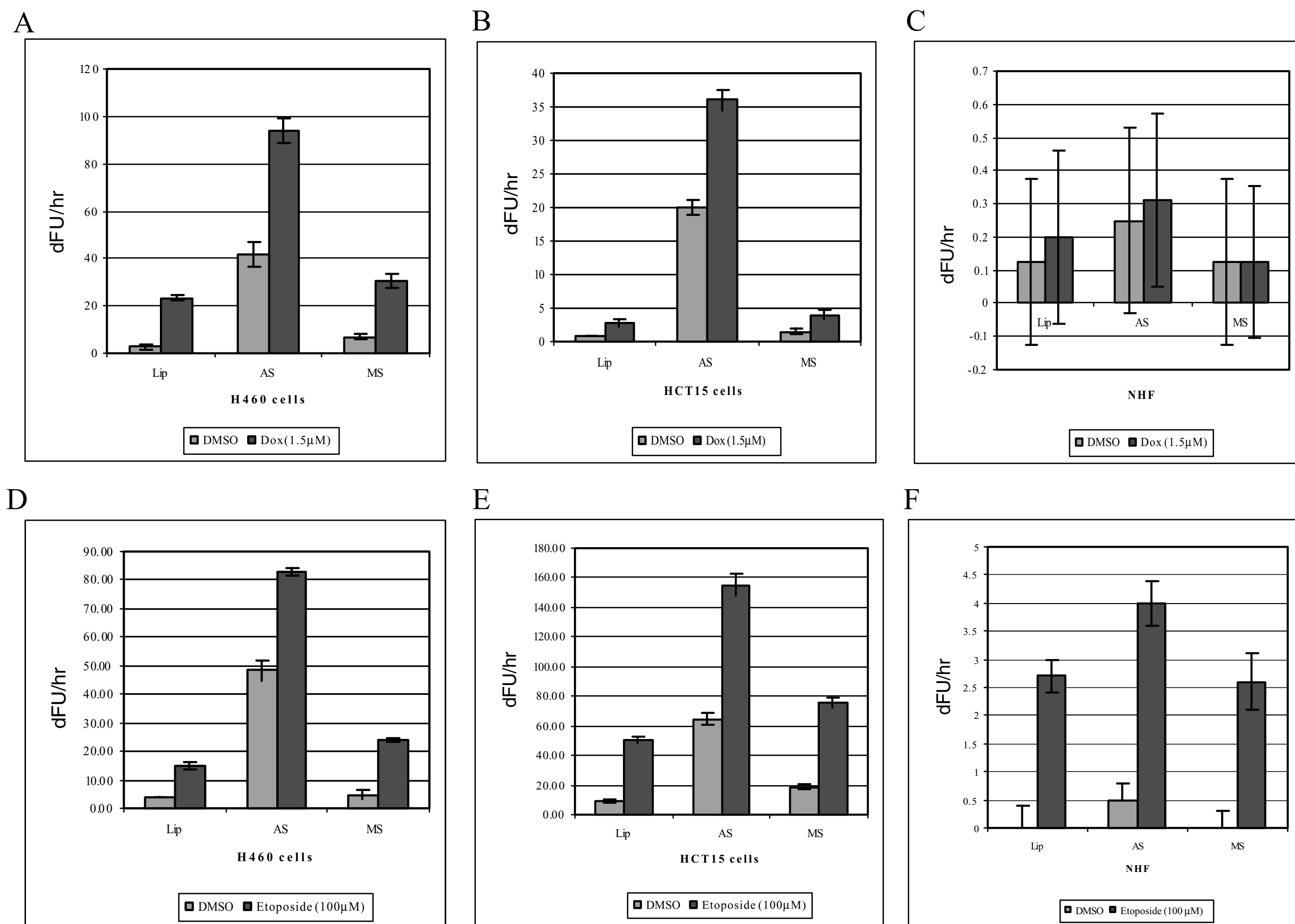

Figure 7. *Akt1 AS sensitized cancer cells to chemotherapeutic drugs. At day 0, 20,000 of H460 or NHF, or 40,000 of HCT15 cells (per well) were plated into 96-well Costar plates. At day 1, cells were transfected with 250 nM Akt1 AS, or MS, or lipofectin control as described in Materials and Methods section. After 24 hours, 1.5 µM doxorubicin was added to HCT15 cells for 15 hours (B), to H460 cells for 8 hours (A), and to NHF cells for 24 hours (C) followed by caspase activity assay as described in Materials and Methods. In another experiment, 24 hours after transfection, 100 µM etoposide was added to H460 (D), or HCT-15 (E), or NHF cells (F) for 24 hours followed by caspase activity assay as described in Materials and Methods. Data are representative of two independent experiments with four replicates for each point. Shown is the mean ± SD of the four replicates.*

unpublished data). In addition to Akt1, Akt2 has been shown to phosphorylate Bad *in vivo* [36]. Thus, it is difficult to differentiate the function of individual Akt isoform by overexpressing the protein in cells. To investigate the role of Akt1 in cancer cells, we tested the effect Akt1 antisense in several different cancer cell lines including a colon cancer cell line (HCT-15), a lung carcinoma cell line (H460), a pancreatic cancer cell line (MiaPaCa-2), a cervix adenocarcinoma cell line (HeLa), and a fibrosarcoma cell line (HT1080). Akt1 AS treatment greatly reduced the growth of these tumor cells in both anchorage-dependent (Figure 3) and anchorage-independent manner (Figure 4). It also induced apoptosis in all these cancer cells (Figure 5). These cancer cell lines are from different tissues and carry various gene mutations such as Ras and p53 mutations [37–39]. The fact that Akt1 AS targeting Akt1 greatly inhibited the proliferation of these tumor cells suggests that Akt1 plays an important role in the malignant phenotype of these cells. Although Akt1 MS oligo did not cause any reduction of Akt1 protein, it did show some nonspecific toxicity as indicated in Figures 3–5*B*. However, the nonspecific toxicity has very little effect on the apoptosis of these cancer cells 24 hours after transfection (Figure 5*A* and data not shown).

The high protein sequence homology and similar substrate specificity [9,36] among Akts raises the possibility that the three Akts may be able to compensate each other. The above statement is supported by the observation that Akt1 AS oligo had no effect on the growth or apoptosis of Panc1 cells where Akt2 is overexpressed (data not shown). The redundant function of Akts might also explain that Akt1 AS treatment on PC3 cells did not induce apoptosis although the Akt1 protein level can be reduced by more than 50% (data not shown). Because there is more Akt3 than Akt1 in PC3 cells [29], the reduction of Akt1 by Akt1 AS could be compensated by Akt3.

Given the fact that constitutively activated Ras resulted in the activation of PI3-kinase/Akt pathway in the cancer cells tested here (MiaPaCa-2, HT1080, HCT-15, and H460) [40], Akt may play a very important role in the survival of these cancer cells. Indeed, downregulation of Akt1 induced them to undergo apoptosis (Figures 5 and 7), even though we did not detect high levels of constitutively active Akts in

these cells (data not shown). The process is probably due to the dephosphorylation of pro-apoptotic proteins such as Bad, caspase-9 or forkhead transcription factors after Akt1 protein reduction. Although we failed to identify the phosphorylation status of Bad and AFX in these cells through Western blot analysis (antibodies from New England BioLabs), we were able to detect the upregulation of p27 in H460 and MiaPaCa-2 cells treated with Akt1 AS (Liu X, unpublished data), suggesting the dephosphorylation of AFX [41] in response to Akt1 protein reduction.

Interestingly, Akt1 AS treatment had no discernable effect on the growth and survival of normal cells such as NHF, FBM, and 184B5 cells (Figure 6). Although more normal cell lines need to be tested, these data suggest that a specific inhibitor for Akt1 may be a useful chemotherapeutic agent. The tumor cell–specific effects of Akt1 inhibition could be due to the fact that cancer cells are more vulnerable to Akt1 protein reduction than normal cells because some cancer cells are more dependent on Akt1.

Akt1 AS Sensitized Cancer Cells to Typical Chemotherapeutic Agents

Doxorubicin and etoposide are topoisomerase II inhibitors that have been shown to induce apoptosis in a number of tumor cell lines [42,43]. These agents have also been used in combination with other agents in the treatment of certain tumors [44]. An advantage of combination chemotherapy is that certain combinations have synergistic effects when compared with either agent alone [45,46]. Our data show that downregulation of Akt1 alone is sufficient to sensitize H460 and HCT15 cells to doxorubicin or etoposide induced apoptosis (Figure 7). It suggests the possibility for the combination treatment of cancer using Akt1 specific inhibitors and cytotoxic drugs such as etoposide or doxorubicin in future clinical applications.

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