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Aldo-keto Reductase Family 1 Member B10 Promotes Cell Survival by Regulating Lipid Synthesis and Eliminating Carbonyls*[S]

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Aldo-keto reductase family 1 member B10 (AKR1B10) is primarily expressed in the normal human colon and small intestine but overexpressed in liver and lung cancer. Our previous studies have shown that AKR1B10 mediates the ubiquitin-dependent degradation of acetyl-CoA carboxylase-α. In this study, we demonstrate that AKR1B10 is critical to cell survival. In human colon carcinoma cells (HCT-8) and lung carcinoma cells (NCI-H460), small-interfering RNA-induced *AKR1B10* silencing resulted in caspase-3-mediated apoptosis. In these cells, the total and subspecies of cellular lipids, particularly of phospholipids, were decreased by more than 50%, concomitant with 2–3-fold increase in reactive oxygen species, mitochondrial cytochrome *c* efflux, and caspase-3 cleavage. *AKR1B10* silencing also increased the levels of α,β-unsaturated carbonyls, leading to the 2–3-fold increase of cellular lipid peroxides. Supplementing the HCT-8 cells with palmitic acid (80 μM), the end product of fatty acid synthesis, partially rescued the apoptosis induced by *AKR1B10* silencing, whereas exposing the HCT-8 cells to epalrestat, an AKR1B10 inhibitor, led to more than 2-fold elevation of the intracellular lipid peroxides, resulting in apoptosis. These data suggest that AKR1B10 affects cell survival through modulating lipid synthesis, mitochondrial function, and oxidative status, as well as carbonyl levels, being an important cell survival protein.

Aldo-keto reductase family 1 member B10 (AKR1B10,[2] also designated aldose reductase-like-1, ARL-1) is primarily expressed in the human colon, small intestine, and adrenal gland, with a low level in the liver (1–3). However, this protein is overexpressed in hepatocellular carcinoma, cervical cancer, lung squamous cell carcinoma, and lung adenocarcinoma in smokers, being a potential diagnostic and/or prognostic marker (1, 2, 4–6).

The biological function of AKR1B10 in the intestine and adrenal gland, as well as its role in tumor development and progression, remains unclear. AKR1B10 is a monomeric enzyme that efficiently catalyzes the reduction to corresponding alcohols of a range of aromatic and aliphatic aldehydes and ketones, including highly electrophilic α,β-unsaturated carbonyls and antitumor drugs containing carbonyl groups, with NADPH as a co-enzyme (1, 7–12). The electrophilic carbonyls are constantly produced by lipid peroxidation, particularly in oxidative conditions, and are highly cytotoxic; through interaction with proteins, peptides, and DNA, the carbonyls cause protein dysfunction and DNA damage (breaks and mutations), resulting in mutagenesis, carcinogenesis, or apoptosis (10, 13–19). AKR1B10 also shows strong enzymatic activity toward all-*trans*-retinal, 9-*cis*-retinal, and 13-*cis*-retinal, reducing them to the corresponding retinols, which may regulate the intracellular retinoic acid, a signaling molecule modulating cell proliferation and differentiation (6, 20–23). In lung cancer, AKR1B10 expression is correlated with the patient smoking history and activates procarcinogens in cigarette smoke, such as polycyclic aromatic hydrocarbons, thus involved in lung tumorigenesis (24–26).

Recent studies have shown that in breast cancer cells, AKR1B10 associates with acetyl-CoA carboxylase-α (ACCA) and blocks its ubiquitination and proteasome degradation (27). ACCA is a rate-limiting enzyme of *de novo* synthesis of long chain fatty acids, catalyzing the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA (28). Long chain fatty acids are the building blocks of biomembranes and the precursor of lipid second messengers, playing a critical role in cell growth and proliferation (29, 30). Therefore, ACCA activity is tightly regulated by both metabolite-mediated allosteric mechanisms and phosphorylation-dependent mechanisms; the latter are controlled by multiple hormones, such as insulin, glucagon, and growth factors (31–33). ACCA activity is also regulated through physical protein-protein interaction. For instance, breast cancer 1 (BRCA1) protein associates with the ACCA and blocks its Ser^{79} residue from dephosphorylation (34, 35). The AKR1B10-mediated regulation on ACCA stability represents a novel regulatory mechanism, and this current study eluci-

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[2] The abbreviations used are: KR1B10, aldo-keto reductase family 1 B10; AKR1B1, aldo-keto reductase family 1 B1; ACCA, acetyl-CoA carboxylase-α; AR, aldose reductase; siRNA, small-interfering RNA; PBS, phosphate-buffered saline; PARP, poly(ADP-ribose) polymerase; CM-H2DCFDA, 5-(and-6)-chloromethyl-2′7′-dichlorodihydrofluorescein diacetate acetyl ester; BSA, bovine serum albumin.

dated the biological significance of this regulation. The results show that AKR1B10 promotes cell survival via modulating lipid synthesis, mitochondrial function and oxidative stress, and carbonyl levels.

MATERIALS AND METHODS

Cell Culture and siRNA Introduction—HCT-8, a human colon adenocarcinoma cell line, and NCI-H460, a human lung carcinoma cell line were purchased from the American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium (Hyclone, Logan, UT) containing 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C, 5% CO_2. Scrambled, *AKR1B10* (10), and *ACCA* (36) siRNAs were chemically synthesized (Ambion, Austin, TX) and delivered into HCT-8 and NCI-H460 cells ($3.5 \times 10^{4-5}$ in Opti-MEM I medium) as described previously (10).

AKR1B10 Activity Assay—Cells were lysed on ice in a buffer containing 20 mM NaH_2PO_4 (pH 7.0), 2 mM β-mercaptoethanol, 5 μM leupeptin, and 20 μM phenylmethylsulfonyl fluoride for 30 min followed by centrifugation at 10,000 × *g*, 4 °C for 10 min. Soluble proteins (50 μg) were used for AKR1B10 activity assay in a reaction mixture consisting of 125 mM sodium phosphate (pH 7.0), 0.2 mM NADPH, 50 mM KCl, and 20 mM DL-glyceraldehyde at 35 °C for 10 min. Oxidized NADPH was monitored at 340 nm for enzymatic activity. For epalrestat (BIOMOL, Plymouth Meeting, PA) inhibition, epalrestat at indicated concentrations was added into the reaction mixtures.

Western Blot—Cells were lysed in complete lysis buffer (Roche Diagnostics) followed by centrifugation at 14,000 × *g* for 15 min to collect soluble proteins. To prepare mitochondrial proteins, cells were washed once with ice-cold PBS and resuspended in 5 volumes of lysis buffer A (20 mM Hepes (pH 7.5), 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, and 250 mM sucrose). Cells were homogenized and centrifuged at 750 × *g* for 10 min at 4 °C. Supernatants were then centrifuged at 15,000 × *g* for 20 min at 4 °C to collect cytosolic proteins. The pellets (mitochondria) were lysed with buffer B (10 mM Tris·Cl (pH 7.4), 1 mM EDTA, 1 mM EGTA, 0.15 M NaCl, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM sodium orthovanadate, and 0.5% Triton X-100) followed by centrifugation at 15,000 × *g* for 15 min at 4 °C to collect mitochondrial proteins. Proteins (100 μg for total proteins or 50 μg for mitochondrial) were separated on 12% (8% for ACCA or 16% for cytochrome *c* and caspase-3) SDS-PAGE and then blotted onto nitrocellulose membranes (Bio-Rad) at 260 mA for 3 h or 200 mA for 2 h for cytochrome *c* and caspase-3. After being blocked with 5% skim milk in TBST solution (125 mM NaCl, 25 mM Tris·Cl, pH 8.0, and 0.05% Tween 20) at room temperature for 1 h, membranes were incubated with AKR1B10 and aldose reductase (AR, also named aldo-keto reductase family 1 B1, AKR1B1) antibody generated in our laboratory (27) or ACCA, poly(ADP-ribose) polymerase (PARP), cytochrome *c*, and caspase-3 antibodies (Cell Signaling, Boston, MA) at 4 °C overnight followed by incubation with goat anti-rabbit or goat anti-mouse IgG (1:2000, LI-COR, Lincoln, NE) for 1 h. Antibody binding was detected using the enhanced fluorescence system (LI-COR Bioscience, Lincoln, NE). Protein amounts were corrected by reprobing membranes with β-actin monoclonal antibody (1:20,000, Sigma) or cytochrome *c* oxidase IV antibody (1:1000, Cell Signaling).

Apoptosis—Apoptosis was evaluated by FACScan and enzyme-linked immunosorbent assay (ELISA). For flow cytometry analysis, 72 h after siRNAs were introduced, medium was gently removed, and cells were washed with cold PBS and then trypsinized. Cells in PBS and trypsin digestions were pooled, washed with PBS twice at 1200 rpm for 10 min, and then subjected to immediate propidium iodide and annexin-V fluorescein isothiocyanate staining for 10 min in the dark. FACScan analysis was performed using a FACScan cytometer (BD Biosciences). Enzyme-linked immunosorbent assays of cell death were carried out using the cell death detection ELISA^{PLUS} kit (Roche Applied Science) following the instructions of the manufacturer.

Lipid Synthesis—Cells were pulsed with 1 μCi of [2-^{14}C]acetate (53 mCi/mmol; Amersham Biosciences) per well of 12-well plates for 4 h at 37 °C, 5% CO_2 in complete medium. Total lipids were extracted as described previously (27). Briefly, after being washed with PBS, cells were harvested by trypsinization and suspended in 40 μl of PBS. An aliquot (10 μl) of the cell suspension was used for protein quantitation, and the remainder was mixed vigorously with 20 volumes of chloroform/methanol (2:1, v/v). After incubation on ice for 10 min, debris was removed at 21,000 × *g* for 10 min, and the supernatant was washed with 0.2 volume of distilled water. The organic phase was collected and dried by speed vacuum. Lipids were dissolved in 50 μl of chloroform/methanol (2:1, v/v). An aliquot (10 μl) of extracts was subjected to radioactivity measurements to determine the total fatty acid synthesis. Acetate incorporation into lipid species, including free fatty acids, phospholipids, triglycerides, and cholesterol, was analyzed by TLC. Lipid extracts and appropriate lipid standards (Sigma) were spotted on silica gel (60 Å; Sigma). After being air-dried, plates were developed in hexane/diethyl ether/acetic acid (70/30/1, v/v) to separate neutral lipids or in chloroform/methanol/acetic acid (65/25/10, v/v) to separate phospholipids. The lipid samples and standards were visualized by coloration in staining solution (0.12 M NaCl, 20% methanol, and 300 mg/liter Coomassie Blue). The lipid fractions were resuspended into 50% methanol solution, and radioactivity were measured by scintillation counter (Beckman Instruments). Values were normalized for protein contents (CPM/mg of protein).

Reactive Oxygen Species Detection—Cells (1×10^4/well) were seeded into 96-well culture plates with optical bottoms for 72 h. After being washed once with PBS, cells were incubated with 10 μM 5-(and-6)-chloromethyl-2′7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA, a cell-permeant indicator for reactive oxygen species that is non-fluorescent until the removal of acetate groups by oxidation; Invitrogen) in PBS at 100 μl/well for 30 min. Thereafter, cells were washed three times with ice-cold PBS and then photographed with a fluorescence microscopy at 485 nm for excitation and 535 nm for emission. For quantitation, cells were lysed in 50 μl of 50 mM sodium phosphate (pH 7.5) with 5 mM butylated hydroxytoluene antioxidant. CM-H2DCFDA (10 μM) in PBS with 5 mM butylated hydroxytoluene was used as a blank control. Fluorescent strength was measured at 535 nm with 485 nm for excitation.

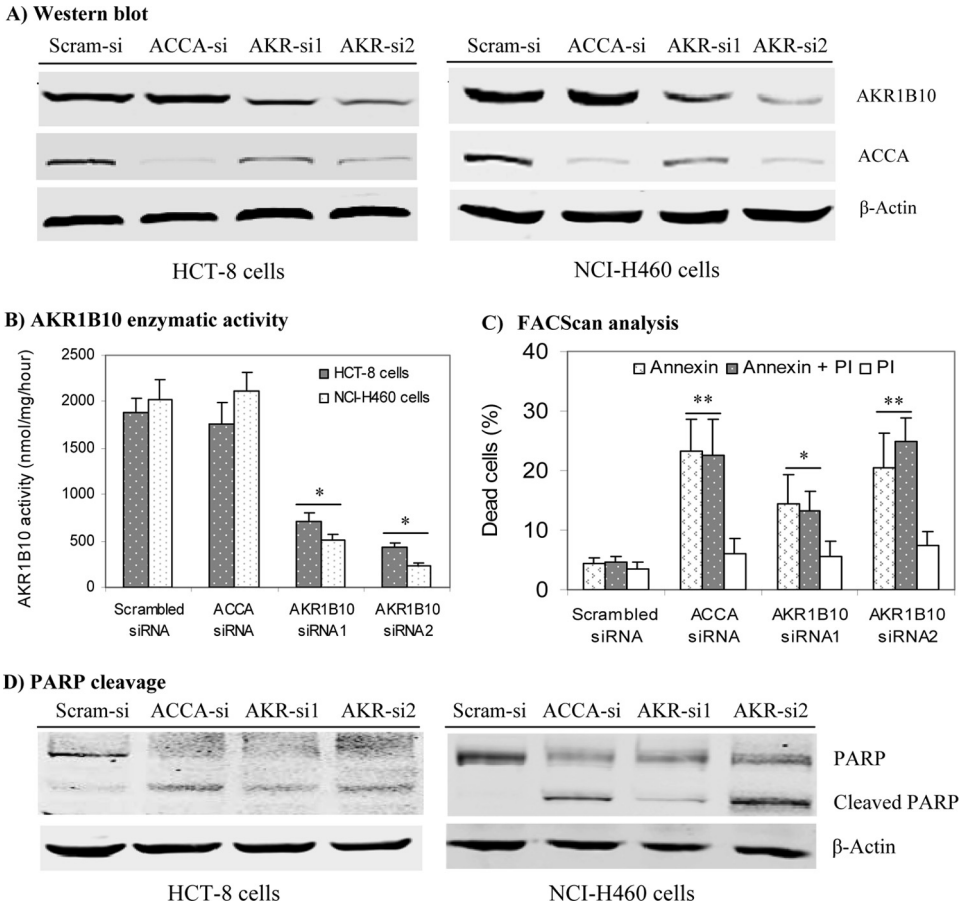

FIGURE 1. **Apoptosis induced by AKR1B10 knockdown.** *AKR1B10* or *ACCA* siRNAs were transiently introduced into the HCT-8 and NCI-H460 cells as described under "Materials and Methods"; 72 h later, cells were subjected to analyses below. *A*, Western blot. 100 μg of soluble proteins were used to examine the AKR1B10 and ACCA protein levels. Scram-si, scrambled siRNA; ACCA-si, *ACCA* siRNA; AKR-si1, *AKR1B10* siRNA1; and AKR-si2, *AKR1B10* siRNA2. *B*, AKR1B10 enzymatic activity. Cell lysates (50 μg of proteins) were used for AKR1B10 activity assays. Results are expressed as oxidized NADPH (nmol)/h/mg of protein. Values represent mean ± S.D. (*error bars*) from three independent measurements. *, $p < 0.01$, when compared with scrambled siRNA control. *C*, FACScan analysis. Cells were collected by trypsinization, stained with annexin-V and propidium iodide (*PI*), and then subjected to FACScan analysis to count the dead cells. Values represent mean ± S.D. (*error bars*) from three independent measurements. *, $p < 0.05$ and **, $p < 0.01$, when compared with scrambled siRNA control. *D*, PARP cleavage. 100 μg of soluble proteins were used for Western blot to detect cleaved PARP from apoptotic cells.

Palmitate Rescues—Palmitate was supplemented with a bovine serum albumin (BSA) complex (36). Briefly, 4 volumes of 4% fatty acid-free BSA (Hyclone) in 0.9% NaCl were mixed with 1 volume of 5 mM palmitate (Sigma) in ethanol and incubated at 37 °C for 1 h to form 1 mM palmitate-BSA complex. Rescues were exerted by adding the palmitate-BSA complex (80 μM) to the cells 24 h after siRNA introduction.

Lipid Peroxidation—Lipid peroxidation was estimated using a lipid peroxidation kit (Bioxytech LPO-586TM; Oxford Biomedical Research, Oxford, MI) that measures the total amounts of the lipid peroxides 4-hydroxynonenal and malondialdehyde following the instructions of the manufacturer.

Statistical Analysis—Student's *t* test or χ-square tests of independence, as appropriate, were used for statistical significance for $p < 0.05$.

RESULTS

Small-interfering RNA-mediated AKR1B10 Silencing Induces Apoptotic Cell Death—AKR1B10 is normally expressed in the intestine but overexpressed in the liver, lung, and breast cancer (1, 4, 27). To understand its biological function in intestinal cells and role in tumor formation and progression, in this study, we evaluated the effects of *AKR1B10* expression on cell survival using lung cancer (NCI-H460) and colon carcinoma (HCT-8) cells. In these cells, AR, a protein highly identical to AKR1B10, is not expressed (supplemental Fig. 1*S*). As shown in Fig. 1*A*, siRNA-mediated *AKR1B10* silencing resulted in significant decrease of ACCA protein in both NCI-H460 and HCT-8 cells, proving our previous finding (27). In these cells, AKR1B10 enzymatic activity was accordingly decreased by ~70–90% when compared with the scrambled siRNA control. An *ACCA*-specific siRNA (36) was used as a control, which effectively down-regulated ACCA but had no effects on AKR1B10 protein and enzymatic activity (Fig. 1, *A* and *B*). The effects of *AKR1B10* silencing on cell survival were assessed by annexin-V staining and FACScan analysis. Results showed that *AKR1B10* silencing resulted in a significant increase of early (annexin-V-positive) and late (both annexin-V-positive and propidium iodide (*PI*)-positive) apoptotic cells (Fig. 1*C*, *right panel*) when compared with the scrambled siRNA control. This was confirmed by PARP cleavage detected by Western blot (Fig. 1*D*). As reported previously in prostate and breast cancer cells (30, 36), *ACCA* silencing also led to apoptosis of the lung (NCI-H460) and colon (HCT-8) cancer cells (Fig. 1, *C* and *D*).

AKR1B10 Silencing Results in Decrease in Lipid Synthesis and Increase in Lipid Peroxides—AKR1B10 detoxifies reactive carbonyls and regulates ACCA stability (9, 10, 12, 27). ACCA is a rate-limiting enzyme of the fatty acid biosynthesis, the precursor of phospholipids essential for biomembranes (30, 37). To understand the molecular/biochemical basis of the cell death induced by *AKR1B10* silencing, we examined lipid synthesis and found that the *AKR1B10* knockdown resulted in more than 50% of decrease in the total cellular lipids and subspecies, particularly in phospholipids, in the HCT-8 and NCI-H460 cells (Fig. 2*A*). Triglyceride and free acid levels also decreased ($p < 0.05$), but cholesterol synthesis did not alter significantly as reported previously (35, 36). Lipid synthesis was decreased more in the *ACCA* silencing cells than in *AKR1B10* silencing, but statistical significance was not detected ($p > 0.05$).

AKR1B10 effectively eliminates cellular lipid peroxides, such as 4-hydroxynonenal (9, 12). Therefore, we further examined

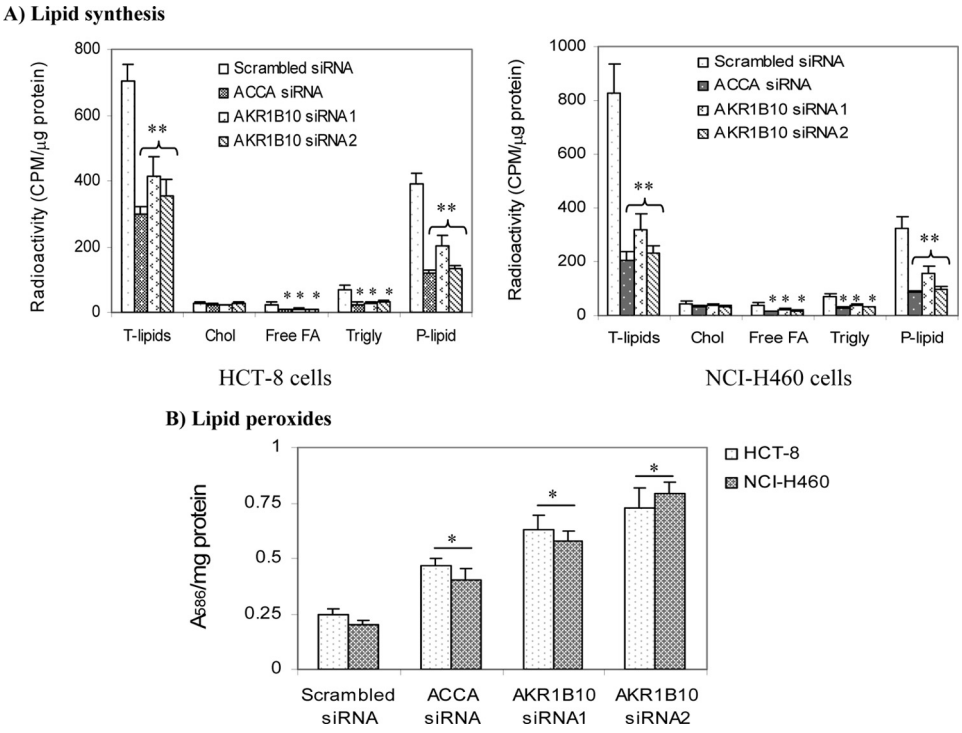

FIGURE 2. **Lipid synthesis and lipid peroxides in *AKR1B10* silencing cells.** *AKR1B10* or *ACCA* silencing in the HCT-8 and NCI-H460 cells was triggered by synthesized siRNAs, and 72 h later, the cells were subjected to the analyses below. *A*, lipid synthesis. Total and subspecies of lipids were measured as described under "Materials and Methods." Values represent mean ± S.D. (*error bars*) from three independent measurements. *, $p < 0.05$ and **, $p < 0.01$, when compared with scrambled siRNA control. *T-lipid*, total lipid; *Chol*, cholesterol; *FA*, fatty acids; *Trigly*, triglycerides; *P-lipid*, phospholipids. *B*, lipid peroxides. Lipid peroxide 4-hydroxynonenal was measured using a lipid peroxidation kit. Values represent mean ± S.D. (*error bars*) from three independent measurements. *, $p < 0.05$, when compared with scrambled siRNA control.

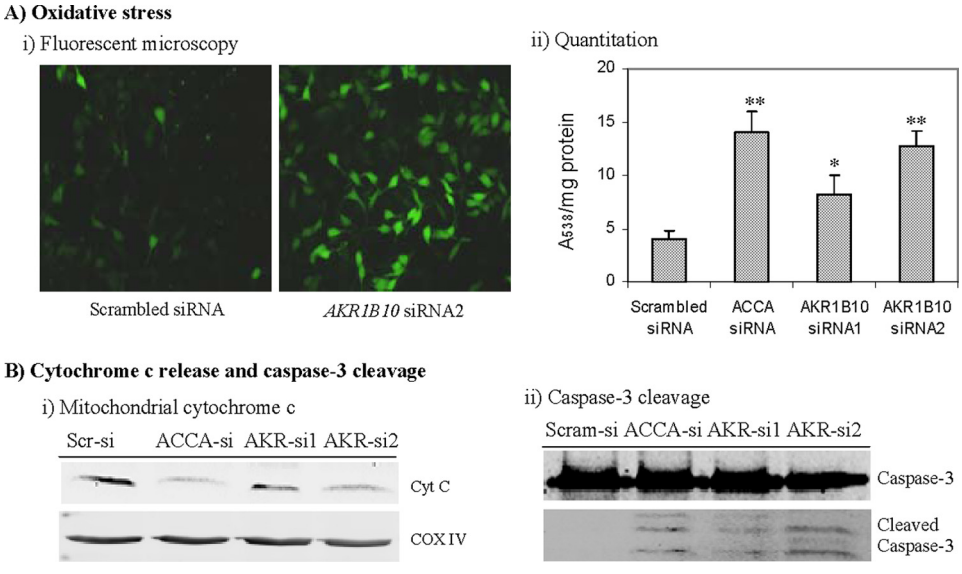

FIGURE 3. **Oxidative stress and cytochrome *c* efflux induced by *AKR1B10* silencing.** *AKR1B10* or *ACCA* silencing in the HCT-8 cells was induced with synthesized siRNAs as described under "Materials and Methods"; 72 h later, cells were subjected to oxidative stress and cytochrome *c* efflux analyses. *A*, oxidative stress. After being washed with PBS, cells were incubated with CM-H2DCFDA (10 μM) at 100 μl/well for 30 min. Photographs (*panel i*) were taken under a fluorescence microscopy with 485 nm for excitation and 535 nm for emission. Quantitation (panel *ii*) was carried out by lysing the cells with 50 μl of 50 mM PBS (pH 7.5) containing 5 mM butylated hydroxytoluene antioxidant. Fluorescent strength was measured at 535 nm with 485 nm for excitation. Values represent mean ± S.D. (*error bars*) from three independent measurements. *, $p < 0.05$ and **, $p < 0.01$, when compared with scrambled siRNA control. *B*, cytochrome *c* release and caspase-3 activation. *Panel i*, mitochondrial cytochrome *c* (*Cyt C*). Mitochondrial proteins (50 μg of each) were used to assess the cytochrome *c* levels by Western blot. The decrease of cytochrome *c* in mitochondria suggests increase in efflux. *Panel ii*, caspase-3 cleavage. Cytosolic proteins (100 μg of each) were used for Western blot to assess the cleavage of caspase-3. *COX IV*, cytochrome *c* oxidase IV; *Scr-si*, scrambled siRNA; *ACCA-si*, *ACCA* siRNA; *AKR-si1*, *AKR1B10* siRNA1; *AKR-si2*, *AKR1B10* siRNA2.

the lipid peroxides in the *AKR1B10* silencing cells, and as shown in Fig. 2*B*, *AKR1B10* silencing led to the 2–3-fold increase of the intracellular lipid peroxides when compared with the scrambled siRNA control. Lipid peroxides in *ACCA* silencing cells were also elevated by ~80% ($p < 0.05$), which may be due to enhanced lipid peroxidation (see below).

AKR1B10 Knockdown Results in Mitochondrial Impairments and Oxidative Stress—Phospholipids are the basic components of the biomembranes of the cells and organelles (30), and therefore, in *AKR1B10* silencing cells, reduced phospholipids may affect the integrity of mitochondrial membranes, thus resulting in oxidative stress and cell apoptosis. As shown in Fig. 3*A*, reactive oxygen species in the HCT-8 cells with *AKR1B10* silencing were indeed enhanced by 2–3 times when compared with the scrambled siRNA control, indicating the impairments of mitochondrial potentials. This damage was confirmed by a mitochondrial cytochrome *c* efflux study (Fig. 3*B*). The mitochondrial cytochrome *c* levels in *AKR1B10* silencing cells were significantly decreased, and the released cytochrome *c* in turn activated caspase-3, triggering apoptosis. Similar results were observed in the NCI-H460 cells (data not shown).

Palmitic Acid (16:0) Partially Rescues Cell Death Induced by AKR1B10 Knockdown—The data above suggest that *AKR1B10* silencing may deplete the cellular phospholipids, thus leading to mitochondrial lesions, cytochrome *c* release, and thereby caspase-3-mediated apoptosis. To verify this hypothesis, we further performed a palmitic acid rescue study. Palmitic acid (16:0) is the end product of the fatty acid synthesis pathway and a precursor of various cellular lipids (30, 37). In this experiment, palmitic acid (80 μM) was added as a BSA complex to the cells with *AKR1B10* or *ACCA* silencing, and the cell death was quantitatively measured

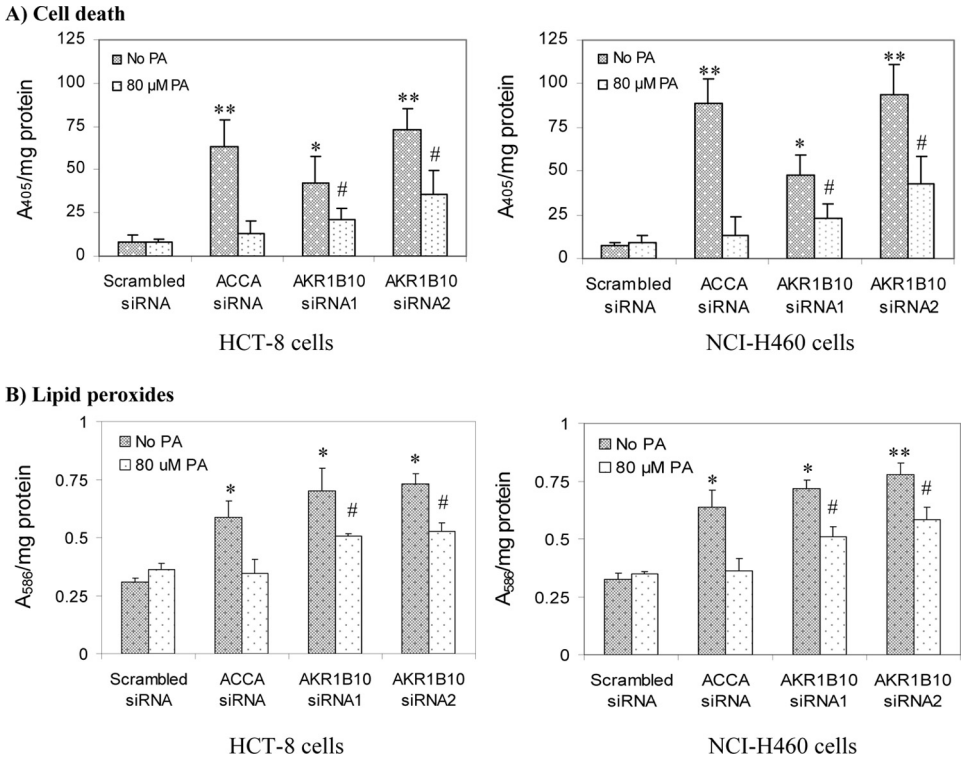

FIGURE 4. **Palmitate acid (*PA*) rescues.** *AKR1B10* or *ACCA* silencing in the HCT-8 and NCI-H460 cells was triggered using synthesized siRNAs and palmitate (80 μM) was supplemented as fetal bovine serum complexes 24 h after siRNAs were introduced. After continuous incubation for 48 h, cells were collected for cell death analysis using a cell death detection ELISA^{PLUS} kit (*A*) and for lipid peroxide analysis using a lipid peroxidation kit (*B*). Values represent mean ± S.D. (*error bars*) from three independent measurements. *, $p < 0.05$ and **, $p < 0.01$, when compared with scrambled siRNA control. #, $p < 0.05$, when compared with the scrambled siRNA control supplemented with palmitic acid.

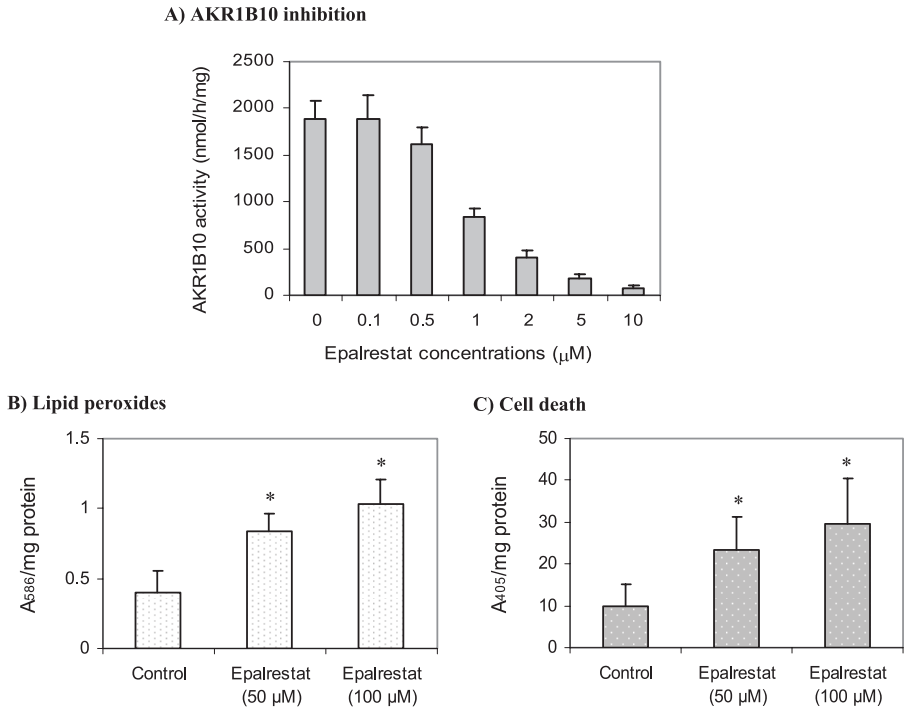

FIGURE 5. **Lipid peroxide accumulation and apoptosis induced by epalrestat.** *A*, epalrestat inhibitory activity. Crude cell lysates from the HCT-8 cells were used to measure the inhibitory activity of epalrestat toward AKR1B10 as described under "Materials and Methods." *B*, lipid peroxides. In the presence of epalrestat (50 or 100 μM) for 24 h, cellular 4-hydroxynonenal was measured using a lipid peroxidation kit. *C*, cell death. Apoptosis induced by epalrestat (100 μM) for 24 h was estimated using cell death detection ELISA^{PLUS} kit. Values represent mean ± S.D. (*error bars*)from three independent measurements. *, $p < 0.05$, when compared with the control cells.

by an ELISA-based cell death detection kit. As shown in Fig. 4*A*, supplementing the cells with palmitic acid (80 μM) partially rescued the apoptosis of the HCT-8 and NCI-H460 cells induced by *AKR1B10* silencing, whereas the palmitic acid rescue was almost complete in the *ACCA* silencing cells. Similarly, palmitic acid supplementation recovered the cellular lipid peroxide levels in part in *AKR1B10* silencing cells but completely in *ACCA* silencing cells (Fig. 4*B*).

AKR1B10 Inhibitor Epalrestat Elevates Cellular Lipid Peroxides and Induces Apoptosis—The capability of palmitic acid to partially rescue cellular lipid peroxide levels and cell death induced by *AKR1B10* silencing (Fig. 4) suggests that AKR1B10 also affects cell survival through eliminating cellular carbonyls. To prove this hypothesis, an AKR1B10 inhibitory study was performed. It has been reported that some aldose reductase inhibitors have inhibitory activity to AKR1B10 (23, 38). In this study, we assessed the inhibitory activity of a new AR inhibitor, epalrestat, and evaluated its effect on the cellular lipid peroxides and cell survival. As shown in Fig. 5*A*, epalrestat effectively inhibited the AKR1B10 activity with an IC_{50} at ~0.8 μM when whole cell lysates from the HCT-8 cells were used as the sources of the AKR1B10. Exposing the HCT-8 cells to 50 or 100 μM epalrestat resulted in more than the 2-fold increase of cellular lipid peroxides (Fig. 5*B*) and ~3-fold elevation of cell death when compared with the vehicle control (Fig. 5*C*). AKR1B10 protein levels and lipid synthesis were not affected by epalrestat (supplemental Fig. 2*S*), indicating that the cell death induced by epalrestat is attributed to increased intracellular carbonyls. Note that AR is not detectable in these cells (supplemental Fig. 1*S*).

DISCUSSION

Lipogenesis is an important cellular event, providing cells with diverse lipids for cell growth, proliferation, and signaling (30, 39, 40).

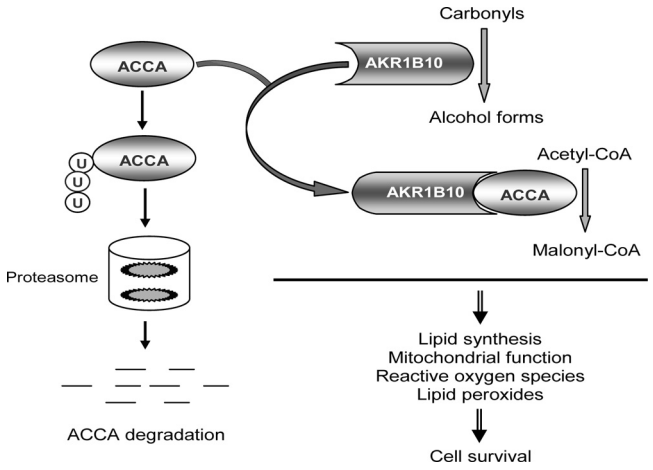

FIGURE 6. **Hypothetical model for the modulation of cell survival by AKR1B10.** ACCA is degraded through COP-1 E3 ubiquitin ligase-mediated ubiquitination-proteasome pathway (45). AKR1B10 blocks ubiquitin-dependent degradation of the ACCA and thus regulates lipid synthesis, mitochondrial function, oxidative, status and lipid peroxidation; AKR1B10 also reduces carbonyls to less toxic alcohol forms, blocking their cytotoxicity. Through these two independent pathways, AKR1B10 promotes cell survival.

Fatty acid synthesis includes two enzymatic reactions. ACCA catalyzes the carboxylation of acetyl-CoA to form malonyl CoA followed by fatty acid synthase-catalyzed elongation of the acyl chain with malonyl-CoA as an acetate moiety donor (30). ACCA is the rate-limiting enzyme in the fatty acid synthesis pathway, and AKR1B10 mediates its degradation through ubiquitination-proteasome pathway in breast cancer cells (27). This study explored the biological significance of this novel regulation, and our results demonstrated that AKR1B10 promotes cell growth and survival by regulating lipid synthesis and carbonyl levels in the lung (NCI-H460) and colon (HCT-8) cancer cells.

AKR1B10 silencing resulted in apoptosis of the HCT-8 and NCI-H460 cells. There may be two mechanisms involved in the *AKR1B10* silencing-induced apoptosis. The knockdown of AKR1B10 resulted in ACCA protein reduction and fatty acid synthesis decrease that depleted cellular phospholipids and led to mitochondrial membrane impairments, cytochrome *c* release, and caspase-3 activation, triggering the apoptosis (Figs. 2 and 3). In addition, the increase of cellular carbonyl levels induced by AKR1B10 knockdown may also contribute to the cell death (Fig. 2). In *AKR1B10* silencing cells, the cellular capability of eliminating cytotoxic carbonyls was weakened due to AKR1B10 knockdown (1, 9, 12), and also, lipid peroxidation was enhanced because of mitochondrial lesions and resultant oxidative stress (14, 41), both of which resulted in the increase of cellular carbonyls. In turn, the oxidative stress and cumulated carbonyls may further aggravate mitochondrial membrane lesions, creating a vicious loop (42, 43).

Epalrestat is an AR inhibitor developed for diabetic clinics (44). Our data showed that epalrestat efficiently inhibits AKR1B10 activity with IC_{50} at 0.8 μM; exposing the HCT-8 cells to epalrestat at 50 or 100 μM significantly enhanced the cellular lipid peroxide (carbonyl) levels and resulted in cell death (Fig. 5). In these cells, AKR1B10 protein levels and lipid synthesis were not changed (supplemental Fig. 2*S*), indicating that AKR1B10 inhibition leads to carbonyl-induced cell death. Note that AR is not expressed in the HCT-8 and NCI-H460 cells (supplemental Fig. 1*S*), excluding its interference with the data interpretation.

ACCA is degraded through COP-1 E3 ubiquitin ligase-mediated ubiquitination-proteasome pathway (45), and epoxomicin, a proteasome inhibitor, can block the ACCA degradation induced by *AKR1B10* silencing (27). This study shows that AKR1B10 knockdown depletes cellular lipid species, impairs mitochondrial potentials, and thus causes oxidation stress and carbonyl lesions. Therefore, it is possible that AKR1B10 modulates the cell survival through two independent pathways, as illustrated in Fig. 6. AKR1B10 mediates ACCA stability through physical association and thus affects fatty acid/lipid synthesis, mitochondrial function, and oxidative status; AKR1B10 also eliminates the cellular carbonyls, blocking carbonyl-induced cell damage. Therefore, AKR1B10 may be a critical cellular protection protein.

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