Research Article

Acyl-Coenzyme A–Binding Protein Regulates Beta-Oxidation Required for Growth and Survival of Non–Small Cell Lung Cancer

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Abstract
We identified acyl-coenzyme A–binding protein (ACBP) as part of a proteomic signature predicting the risk of having lung cancer. Because ACBP is known to regulate β-oxidation, which in turn controls cellular proliferation, we hypothesized that ACBP contributes to regulation of cellular proliferation and survival of non–small cell lung cancer (NSCLC) by modulating β-oxidation. We used matrix-assisted laser desorption/ionization-imaging mass spectrometry (MALDI-IMS) and immunohistochemistry (IHC) to confirm the tissue localization of ACBP in pre-invasive and invasive NSCLCs. We correlated ACBP gene expression levels in NSCLCs with clinical outcomes. In loss-of-function studies, we tested the effect of the downregulation of ACBP on cellular proliferation and apoptosis in normal bronchial and NSCLC cell lines. Using tritiated-palmitate (3H-palmitate), we measured β-oxidation levels and tested the effect of etomoxir, a β-oxidation inhibitor, on proliferation and apoptosis. MALDI-IMS and IHC analysis confirmed that ACBP is overexpressed in pre-invasive and invasive lung cancers. High ACBP gene expression levels in NSCLCs correlated with worse survival (HR=1.73). We observed a 40% decrease in β-oxidation and concordant decreases in proliferation and increases in apoptosis in ACBP-depleted NSCLC cells as compared with bronchial airway epithelial cells. Inhibition of β-oxidation by etomoxir in ACBP-overexpressing cells produced dose-dependent decrease in proliferation and increase in apoptosis (P=0.01 and P<0.001, respectively). These data suggest a role for ACBP in controlling lung cancer progression by regulating β-oxidation. Cancer Prev Res; 7(7); 748–57. ©2014 AACR.

Introduction
Early detection efforts in our laboratory have led to the discovery by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) of acyl–coenzyme A–binding protein (ACBP) as part of a 6-protein signature predicting the risk of having lung cancer in individuals with endobronchial lesions (1,2). This novel observation prompted us to investigate its role in lung cancer progression. The ACBP gene encodes a 90–amino acid protein with molecular mass of 10 kDa that binds medium to long-chain acyl-CoA esters (C12–C22) with high affinity and specificity (3,4). ACBP was formerly known as the diazepam-binding inhibitor (DBI) by its ability to displace diazepam from its binding sites on type A gamma-aminobutyric acid receptors (GABA_A receptors; ref. 5). ACBP induces steroidogenesis in isolated adrenal mitochondria, inhibits glucose-induced insulin secretion from the pancreas, induces medium-chain acyl-CoA ester synthesis, and affects cell growth (6–12). Mostly cytosolic, ACBP is localized to the endoplasmic reticulum (ER) and Golgi apparatus (13). Fatty acyl-CoAs (ACBP’s substrate) are transported across the inner mitochondrial membrane by carnitine and play an important role in a host of cellular processes such as phospholipid synthesis, protein kinase C activation (PKC), and β-oxidation (7,9,12,14). Cancer cells favor alternative pathways to meet the increased nutritional demand of cell growth (15). The mitochondrial metabolism can be reprogrammed to meet the macronutrient demand of sustained cellular growth (15). The catabolism or β-oxidation of acyl-CoAs is an important process in fulfilling these cellular energy requirements during metabolic stress. The process involves acyl-CoA moieties being broken down in the mitochondria to generate acetyl-CoA. The acetyl-CoA can...
then serve as the substrate for the tricarboxylic acid (TCA) cycle for the generation of ATP. Carnitine palmitoyl transferase 1 (CPT-1) is the key regulatory enzyme mediating the transport of acyl-CoAs with carnitine into the mitochondria. CPT-1 interacts with the acyl-CoA/ACBP complex, and the activity of CPT-1 is correlated with the concentration of ACBP-bound acyl-CoAs, but not with levels of free acyl-CoAs (16). The β-oxidation pathway is a dominant bioenergetic pathway in prostate cancer which increases fatty acid utilization to provide increased ATP levels; however, little is known about its role in lung cancer (17). We therefore hypothesize that ACBP regulates proliferation and survival of non–small cell lung cancer (NSCLC) by modulating β-oxidation. Determining the role of ACBP in lung cancer may enhance our understanding of lung cancer progression and establish its implications in diagnosis and treatment.

Material and Methods

MALDI–time-of-flight imaging MS (MALDI-TOF-IMS) proteomic analysis

Bronchial biopsy specimens were frozen in liquid nitrogen immediately after collection under an Institutional Review Board (IRB)-approved protocol, embedded in Tissue-Tek Optimal Cutting Temperature compound (iMEB), and stored at −80°C. To determine the location of protein expression by MS, we followed protocols described in earlier work (1, 18–20). Briefly, a section from a unique bronchial tissue specimen containing progressive histologic stages of NSCLC development, including normal bronchial epithelium, moderate dysplasia, severe dysplasia, carcinoma in situ (CIS), and invasive cancer, was cut at 12 μm and immediately thaw-mounted on conductive indium-tin oxide–coated glass slides (Delta Technologies Ltd). A sequential section was cut and mounted onto a charged glass slide and stained with hematoxylin and eosin (H&E; Fig. 1A). The acoustic robotic microspotter was used to print matrix (sinapinic acid prepared at 1 mg/mL in 60/40-acetonitrile/0.1% trifluoroacetic acid) in an array format with a center-to-center distance of 300 μm covering the entire surface of the section (Fig. 1B). A total of 28 droplets of matrix were collected at each array coordinate to form the MALDI spots. MALDI-IMS data were acquired on an Applied Biosystems DE-STR TOF MS, equipped with a nitrogen laser (N₂, 337 nm) operated at a repetition rate of 20 Hz. Protein MS data were acquired in the linear mode geometry with 25 kV of accelerating potential under optimized delayed extraction conditions, focusing the ions at ~m/z 15,000. Under these conditions, optimal signal resolutions were obtained throughout the studied mass range from 2 to 25 kDa. MALDI-IMS data acquisition was automated using a custom-built plate geometry file corresponding to the printed matrix array. From each matrix spot of the array, a mass spectrum was generated by summing the signals from 250 consecutive laser shots. Ion images were assembled using custom-built software and visualized using Biomap (Novartis). After data acquisition, matrix was removed from the section by immersion in 70% ethanol for 1 minute. The section was then immediately stained with H&E.

Validation of ACBP localization and expression by immunohistochemistry

Five-micrometer tissue sections were cut from formalin-fixed, paraffin-embedded (FFPE) lung tissue blocks, deparaffinized by placing in 3 xylene baths, 10 minutes each, hydrated through a series of ethanol baths of decreasing concentration, and placed in a buffer bath of TBS. Endogenous peroxide was quenched using Dako Peroxidase
Blocking Reagent and nonspecific staining blocked using Dako Serum-Free Protein Block according to manufacturer's instructions. IHC analysis was performed as previously reported by our group (21). Results of the immunohistochemical (IHC) staining were analyzed by a pathologist (R. Eisenberg).

Analysis of overall survival of patients with high ACBP expression

Kaplan–Meier plotter on line survival analysis software (22) was used to test the relevance of changes in gene expression to overall survival (kmplotter.com). The following publically available datasets were used: GSE31210, GSE29013, and GSE4573. Mixed adenocarcinomas and squamous cell carcinomas (SCC), staged 1–3 (411 total samples), were represented. HR (and 95% confidence intervals) and log-rank P are provided in the online application; the association between ACBP and clinical variables was analyzed using a multivariant Cox regression excluding biased arrays.

Cell culture

Human lung cancer cell lines A549, adenocarcinoma, H520, SCC, and the normal human bronchial epithelial (HBE) cell line, 16-HBE (ATCC) were maintained in RPMI-1640 medium (Gibco by Life Technologies) containing 10% heat-inactivated FBS (Gibco by Life Technologies), at 37°C, 100% humidity, and 5% CO2. Cells were tested every 6 months for mycoplasma using the PCR-based MycoAlert PLISS Mycoplasma Detection Kit (Lonza). For cells that had been infected with shRNA lentivirus, cells were maintained in additional media that contained 1.5 μg/mL puromycin (Sigma). Transfected cells were of low-passage number (>15 passages), and further tested for all common murine pathogens by PCR as PARP and cleaved caspase-3 antibodies were from Cell Signaling. All antibodies were used according to manufacturer’s instructions.

Reagents and antibodies

Etomoxir sodium salt were purchased from Sigma Aldrich. ACBP antibody was purchased from Abcam, where as PARP and cleaved caspase-3 antibodies were from Cell Signaling. All antibodies were used according to manufacturers’ dilutions. Puromycin dihydrochloride (Invitrogen) was used at a concentration of 1.5 μg/mL for colony selection.

β-Oxidation assay

β-Oxidation measurements were performed as previously described with slight modifications in cell media (25). Cells were plated in RPMI culture medium containing 10% FBS. Cells were plated in 6-well dishes and allowed to adhere overnight, and media were replaced with defined culture media supplemented with EGF (25 ng/mL) and with 1x growth factor cocktail (Invitrogen) that includes insulin, selenium, and transferrin. HEK293T cells were maintained in DMEM with 10% FBS.

shRNA knockdown

To stably silence ACBP in H520, A549, and 16-HBE cells, 3 individual pGIPZ lentiviral shRNA-ACBP and 1 pGIPZ nonsilencing shRNA lentiviral control vector were purchased from Open Biosystems. Transfection and transduction were conducted according to manufacturer’s instructions or as previously described (23). Briefly, low-passage HEK293T cells were plated and allowed to grow to about 90% confluency. Lentiviral packaging mix and lentiviral vectors were introduced to the cells and allowed to integrate into the genome. Media were changed and 2 days postintegration virus was collected. Virus was then purified by filtering through a 0.4-μm filter. Cells to be infected were plated the day before infection and subsequently incubated with virus in 8 μg/mL polybrene for 6 hours. Puromycin (1.5 μg/mL) was added the following day for selection. After colony selection, ACBP expression in various cell colonies was measured using immunoblotting.

Proliferation assays

Cells were seeded into 24-well plates at a density of 2 × 10⁴ per well. Cells were allowed to adhere overnight, and the next day cells were washed with PBS and 500 μL of supplemented media was added. At the respective time points, 50 μL of CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (MTS) solution was added into each well and the experiment was carried out as per the manufacturer’s instructions (Promega). Briefly, cells were gently shaken, and the plates were incubated at 37°C for 1 hour. The absorbance was detected at 490 nm with a Microplate Reader (Epoch Microplate Spectrophotometer, Biotek). All experiments were repeated 3 times. The WST-1 assay (Roche) was performed as previously reported by our group (24). Briefly, stably transfected H520 cells were seeded in 96-well plates at 1.5 × 10³ cells per well. Cells were allowed to adhere overnight, and the next day cells were washed with PBS and 500 μL of supplemented media was added. WST-1 reagent was used for spectrophotometric quantification of cell proliferation.

ATP assay

ATP measurements were determined using the ATP Colometric/Fluorometric Assay Kit (Abcam). Measurements were performed with the fluorometric assay in 96-well black bottom plates at the 48-hour time point, following the manufacturer’s instructions.
round-bottom snap-top tubes. The remaining assay was performed as indicated in the cited protocol.

**Mitochondrial potential assay**

The lipophilic cation MitoProbe JC-1 Assay Kit (Cayman Chemical) was used to assess the mitochondrial status of NSCLC cells with etomoxir added. JC-1 (Invitrogen) is a lipophilic membrane-permeant cation that selectively enters the mitochondria. JC-1 exists in a monomeric form producing a green fluorescence or an aggregated form producing a red fluorescence upon mitochondrial hyperpolarization. Briefly, cells were plated at a density of 5 × 10^4 cells per 200 mL in 96-well black, clear-bottom plates. Cells were allowed to adhere overnight and subsequently washed. The respective concentrations of etomoxir mixed with supplemented, defined RPMI media were then added. Sixteen hours later, the JC-1 assay was performed, and fluorescence was measured in a Flexstation II plate reader (Molecular Device) with wavelengths of excitation and emission (ex/em) 530/580 nm (‘red’) and then at ex/em 485/535 nm (‘green’). The ratio of green to red reflects the Δψm (membrane potential).

**Statistical analysis**

Statistical analysis for the proliferation assays, β-oxidation assay, ATP assay, and Δψm indicator assay was performed using the GraphPad Prism software (GraphPad Software). Data comparing 2 experimental conditions were analyzed by 2-tailed Student t test. Only results with P < 0.05 or P < 0.01 were considered to be statistically significant. All experimental data are presented as a representative of 3 independent experiments. All treatments within each experiment were performed in quadruplicate wells (proliferation assay, ATP assay, and Δψm indicator assay) or triplicate fashion (β-oxidation assay) and repeated on 3 independent days.

**Results**

**Characterization of ACBP expression and the clinical relevance to NSCLCs**

To further assess the relevance of ABCP in lung cancer progression, we obtained a unique 2-cm tissue sample that displayed varying levels of cancer pathologic progression, including normal bronchial epithelium, moderate dysplasia, severe dysplasia, CIS, and invasive cancer (Fig. 1A). The levels of ACBP expression increased from normal to CIS (Fig. 1C). ACBP has an m/z value of 9,955 and the corresponding MALDI-MS peak amplitude varied in intensity across the spectrum from normal to pre-invasive and invasive tissues (Fig. 1D). We next analyzed ACBP levels in a panel of paired primary lung SCCs, adenocarcinomas, and matched normal adjacent lung tissues by Western blot analysis. We discovered that ACBP is overexpressed in a majority of the SCCs and adenocarcinomas compared with the adjacent normal lung tissue (Fig. 2A and B). To further test whether ACBP is associated with lung cancer progression, we used IHC to stain additional tissue sections of pre-invasive lesions varying in severity to demonstrate an increased staining pattern going in a continuum from normal bronchial epithelium to lung cancer (Fig. 2C). The immunostaining pattern was predominantly cytoplasmic, but exhibited some nuclear localization (Fig. 2C). We also note that the staining in the normal bronchial epithelium is confined to inflammatory cells, pneumocytes, and macrophages (Fig. 2C). Examining mRNA expression data in 411 NSCLC samples (including 241 patients with stage I, 91 with stage II, 23 with stage III disease, and 56 with unknown staging), our multivariate Cox analysis revealed that ACBP expression is correlated with NSCLC stage (HR = 1.9; 95% CI, 1.49–2.41), patient smoking history (HR = 0.53; 95% CI, 0.3–0.95), and overall poor survival in NSCLCs (HR =
high expression (red line) or low expression (black line) of
patients with lung cancer. Overall survival of patients with NSCLCs with
strating both early- and late-stage apoptosis, respectively
cleaved caspase-3 and cleaved PARP in H520 cells demon-
PARP. ACBP downregulation resulted in increases in both
levels of 2 apoptotic markers, cleaved caspase-3 and cleaved
(Fig. 4F). In contrast, ACBP silencing by shRNA had no
noticeable effects on either cleaved caspase-3 or cleaved
PARP in 16-HBE cells (Supplementary Fig. S2C). Together,
these results confirmed that downregulation of ACBP inhib-
its NSCLC proliferation and induces apoptosis in the
cancer cell lines tested.

Fatty acid oxidation is mediated by ACBP in NSCLCs
ACBP is important for a host of biologic responses,
including regulation of the intracellular acyl-CoA pool size
and donation of acyl-CoA esters for β-oxidation (26).
Because cancer cells rely on altered metabolism and repro-
gramming of metabolic pathways for survival (27), we
hypothesized that adenocarcinomas and SCCs rely on
mitochondrial ACBP-mediated β-oxidation for overall sur-
vival. Using tritiated-palmitate (1H-palmitate) at normal
physiological concentrations of 40 μmol/L as a precursor
substrate, we measured β-oxidation levels using scintilla-
tion counts of ion exchange column fragments in H520 and
16-HBE cells. Because β-oxidation increases when cells
undergo metabolic stress, cells were cultured without glu-
cose and we measured β-oxidation levels. In ACBP control
vector cells, we discovered that without glucose there is a
statistically significant increase in β-oxidation [P < 0.05; Fig.
5A]. In contrast, in ACBP-depleted cells, in the presence of
glucose, there is a statistically significant decrease in β-oxi-
dation compared with control cells (P < 0.05; Fig. 5A).
Moreover, we did not observe any significant increase in
β-oxidation in our ACBP-depleted cells when glucose was
removed. This suggests that ACBP mediates β-oxidation in
H520 cells. In contrast to H520, β-oxidation levels were
unaffected in 16-HBE cells upon ACBP depletion (Fig. 5B).
These results indicate that the downregulation of ACBP
affects lung cancer cell metabolism.

Fatty acid oxidation is required for NSCLC growth and survival
To further determine whether β-oxidation is a key player in
this ACBP-mediated regulation of cell proliferation and
survival, we inhibited β-oxidation pharmacologically using
etomoxir, a potent inhibitor of CPT-1 (28). We first con-
firmed that etomoxir could in fact inhibit β-oxidation in
NSCLCs at a relatively low dose, that is, 10 μmol/L (Fig. 5C).
To further elucidate the effect of inhibition of β-oxidation
on NSCLC cells mitochondrial integrity, the membrane-
permeant JC-1 dye was used to quantify alterations in
mitochondrial potential that can serve as an early sign of
apoptosis (29, 30). Using the JC-1 assay, we found a dose-
dependent decrease in mitochondrial potential (Δψm) in
both H520 and A549 cells by etomoxir (Fig. 5E; Supple-
mentary Fig. S3). The effect was more pronounced at lower
concentrations in the H520 cells than in the A549 cells. This
is consistent with the prosurvival role of the β-oxidation
pathway in NSCLCs. To further test the importance of the
β-oxidation pathway on growth and survival, we examined
proliferation when β-oxidation is inhibited. Etomoxir
induced a dose-dependent decrease in proliferation in
H520 cells with intact ACBP (Fig. 5D). The decrease in

Loss of ACBP function reduces NSCLC proliferation and survival
We found that ACBP is differentially expressed in all
NSCLC cell lines tested compared with noncancerous HBE
(16-HBE in Fig. 4A and HIC Supplementary Fig. S1). To
test the hypothesis that high ACBP levels contribute to
NSCLC proliferation and survival, we knocked down ACBP
with shRNA in H520 and A549 cells, 2 NSCLC cell lines
strongly expressing ACBP (Fig. 4A). We used the immor-
talized human bronchial cell line 16-HBE as a control. Two
efficient knockdown clones were generated using single-
colony selection under puromycin. Visually, both shRNA
clones showed efficient (80%) knockdown of ACBP in the 3
cell lines tested (H520 and A549 in Fig. 4B and C and 16-
HBE in Supplementary Fig. S2A). To determine whether
ACBP depletion affected NSCLC proliferation, we measured
cell proliferation in the ACBP shRNA–depleted cells. Silenc-
ing ACBP significantly reduced the proliferation of H520
and A549 (Fig. 4D and E). In contrast, silencing of ACBP in
16-HBE cells had no significant effect on proliferation
(Supplementary Fig. S2B). To determine whether the effects
on cell proliferation are due to apoptosis, we examined the
levels of 2 apoptotic markers, cleaved caspase-3 and cleaved
PARP. ACBP downregulation resulted in increases in both
cleaved caspase-3 and cleaved PARP in H520 cells demon-
strating both early- and late-stage apoptosis, respectively

Figure 3. ACBP overexpression correlates with poor overall survival in
patients with lung cancer. Overall survival of patients with NSCLCs with
high expression (red line) or low expression (black line) of ACBP.
Data are derived from 3 publically available gene expression data sets
(GSE31210, GSE29013, and GSE4573; n = 411) using a mean
expression of 3 probes that match the annotated ACBP transcript.

1.73; 95% CI, 1.19–2.51; Fig. 3, Supplementary Table S1;
ref. 22). Taken together, these results demonstrate that
ACBP is differentially expressed in NSCLC cell lines and
overexpressed in lung cancer and in pre-invasive squamous
lung tissues.

Fatty acid oxidation is required for NSCLC growth and survival
To further determine whether β-oxidation is a key player in
this ACBP-mediated regulation of cell proliferation and
survival, we inhibited β-oxidation pharmacologically using
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induced a dose-dependent decrease in proliferation in
H520 cells with intact ACBP (Fig. 5D). The decrease in

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proliferation observed upon ACBP depletion was more dramatic than that seen with simply decreasing β-oxidation with etomoxir. This hints at other factors that might be influenced by ACBP. To test whether the loss of mitochondrial potential is associated with reduction of ATP, we measured ATP production levels upon ACBP downregulation in H520 cells. We observed that inhibition of β-oxidation in control cells induces a statically significant decrease in ATP levels (Supplementary Fig. S4). We also note both quantitative and qualitative JC-1 dye incorporation, suggesting mitochondrial injury leading to early apoptosis in H520 cells (Fig. 5E and F, respectively). Taken together, these data suggest that ACBP mediates β-oxidation via CPT-1 and that inhibition of the ACBP-β-oxidation pathway has deleterious effects on NSCLC growth and survival (Fig. 6).

Discussion

Here we report for the first time the translational relevance and the biologic function of the lipid-binding protein ACBP in lung cancer progression. Our studies demonstrate by orthogonal proteomic methods that ACBP is overexpressed in lung cancer and along the stages of squamous carcinoma development. We report that high levels of ACBP expression correlate with overall poor patient survival. We show that ACBP contributes to regulation of cellular proliferation and survival of NSCLCs by modulating β-oxidation. This opens the possibility of targeting the ACBP/CPT-1/β-oxidation axis to control NSCLC cell growth. This report advances our understanding of the molecular events underlying lipid homeostasis in lung cancer as a key component of cancer metabolism and pathogenesis (31).

ACBP functions as an intracellular carrier of acyl-CoA ester and is also known as diazepam-binding inhibitor (DBI). ACBP has much greater affinity for acyl-CoA than fatty acid-binding proteins (FABP), a family of proteins also implicated in cancer progression. FABPs have a similar function to ACBP in that they are carrier proteins transporting important lipophilic substances between extra- and intracellular membranes (32, 33). In lung cancer, studies show that liver-FABP (L-FABP) was detected in 60% (120 of 199) of surgically resected lung carcinoma cases; however, there was no association between L-FABP and clinicopathologic data (34). In breast cancer, it has been determined that FABP5 plays a critical role in regulating breast tumorigenesis (35). Moreover, there have been synthetic drugs...
Figure 5. ACBP downregulation decreases β-oxidation in H520 cells. A, downregulation of ACBP by shRNA inhibits β-oxidation in cancer cells. In these experiments, [3H]-palmitate levels in cells expressing control vector (Cntl Vector) are compared with the ACBP shRNA. B, downregulation of ACBP has no significant effect on β-oxidation in 16-HBE cells. The results in A and B were normalized to protein levels. Glucose was removed (-Glc) in both control vector and ACBP-depleted cells to ensure that we were measuring β-oxidation levels. C, decrease in β-oxidation levels using 10 μmol/L of CPT-1 inhibitor, etomoxir, in H520 cells. D, dose-dependent effect of etomoxir on the proliferation in H520 cells. Proliferation was measured by the MTS proliferation assay. E, dose-dependent increase of etomoxir on mitochondrial potential measured. Alterations in mitochondrial potential were measured by JC-1 accumulation in H520 cells. F, qualitative assessment of mitochondrial injury as measured by JC-1 (red, healthy viable cells; green, unhealthy, early apoptotic cells). *, P < 0.05; **, P < 0.001. Error bars denote SD between replicates. Data, representative of 3 separate, independent experiments. CPM, counts per minutes; RFU, relative fluorescent units.

designed to inhibit inflammation that could potentially inhibit substrate binding to the FABPs (36).

Similar to our assertion that ACBP is overexpressed in lung cancer and inflammatory cells, and associated with poorer patient survival, in bladder cancer, a study suggests that ACBP could be predictive of poor survival and of poor response to chemotherapy (37). Lee and colleagues also demonstrated upregulation of the ACBP gene in their DNA microarray dataset of normal, neoplastic, and invasive epithelial ovarian tissues (38). Using IMS, we have a unique opportunity to assess the expression of ACBP in a patient specimen harboring various types of pre-invasive lesions that recapitulate the natural history of squamous carcinoma of the lung. We further validated ACBP expression in independent tissue specimens by IHC. These studies prompted us to further investigate the biologic implications of ACBP overexpression in lung cancer.

We show that ACBP depletion reduces cell proliferation and induces apoptosis in the NSCLC cell lines tested. This observation was strengthened as we demonstrated that ACBP mediates β-oxidation in lung cancer cells. β-Oxidation plays a crucial role in providing the acyl-CoA substrate for the β-oxidation pathway and controlling cell growth. β-Oxidation is the process by which fatty acids are broken down in the mitochondria to generate acetyl-CoA. The acetyl-CoA can serve as the substrate for the TCA cycle and as a substrate for fatty acid synthesis. One of the key regulators of β-oxidation is CPT-1, for which ACBP serves as an endogenous ligand. CPT-1 mediates the transport of acyl-CoAs into the mitochondria for β-oxidation. Moreover, CPT-1 prefers the acyl-CoA complexed with ACBP over free-acyl-CoAs (39). Pharmacologic inhibition of CPT-1 using the drug etomoxir results in ATP depletion and cell death in glioblastomas and sensitizes human leukemia cells to apoptosis (40, 41). Recently, inhibition of CPT-1 protein levels were shown to reduce cell growth in the in vitro models of lung, breast, and prostate cancers and in in vivo models of breast cancer under...
metabolic stressors (42). Our data suggest that the β-oxidation pathway is required to meet macromolecular demands. It has been largely accepted that cancer cells require fatty acid synthesis, coupled with the key enzyme fatty acid synthase for growth and survival (43–47). It may seem counterintuitive that cancer cells would use β-oxidation, a degradation pathway, during their rapid growth and expansion. However, when we consider that the cancer cells can quickly deplete other nutritional sources, such as glucose and glutamine, β-oxidation becomes an ideal source for energy. A recent report demonstrates that β-oxidation is upregulated in alveolar cells isolated from mice exposed to cigarette smoke (48). There may be a true metabolic shift in alveolar type II cells from glucose utilization (glycolysis) to palmitate utilization (β-oxidation) for energy production after cigarette smoke exposure. This is in line with our hypothesis that β-oxidation, regulated by ACBP, is important for lung cancer growth.

In summary, we identified a set of lung cancers overexpressing ACBP that have worse overall survival implicating ACBP in cancer progression. Our results suggest that ACBP plays a role in lung cancer growth and survival by regulating β-oxidation. Understanding of the role of ACBP in lung cancer progression could play a pivotal role in future studies investigating the ACBP/CPT-1/β-oxidation pathway in controlling NSCLC pathophysiology and treatment.

Disclosure of Potential Conflicts of Interest
R.M. Caprioli is the Editor-in-Chief of the Journal of Mass Spectrometry. No potential conflicts of interest were disclosed by the other authors.
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