Aldo-keto Reductase Family 1 B10 Affects Fatty Acid Synthesis by Regulating the Stability of Acetyl-CoA Carboxylase-\(\alpha\) in Breast Cancer Cells*§

Received for publication, September 12, 2007, and in revised form, November 30, 2007 Published, JBC Papers in Press, December 1, 2007, DOI 10.1074/jbc.M70760200

Jun Ma, Ruilan Yan, Xuyu Zu, Ji-Ming Cheng, Krishna Rao, Duan-Fang Liao, and Deliang Cao

From the 4Department of Medical Microbiology, Immunology, and Cell Biology, SimmonsCooper Cancer Institute, and 6Division of Hematology/Oncology, Internal Medicine, Southern Illinois University School of Medicine, Springfield, Illinois 62702 and 5Division of Pharmacoproteomics, Institute of Pharmacy and Pharmacology, Nanhua University School of Life Science and Technology, 28 Changshengxi Road, Hengyang, Hunan 421001, China

Recent studies have demonstrated that aldo-keto reductase family 1 B10 (AKR1B10), a novel protein overexpressed in human hepatocellular carcinoma and non-small cell lung carcinoma, may facilitate cancer cell growth by detoxifying intracellular reactive carbonyls. This study presents a novel function of AKR1B10 in tumorigenic mammary epithelial cells (RAO-3), regulating fatty acid synthesis. In RAO-3 cells, Sephacryl-S 300 gel filtration and DEAE-Sepharose ion exchange chromatography demonstrated that AKR1B10 exists in two distinct forms, monomers (\(~40\) kDa) bound to DEAE-Sepharose column and protein complexes (\(~300\) kDa) remaining in flow-through. Co-immunoprecipitation with AKR1B10 antibody and protein mass spectrometry analysis identified that AKR1B10 associates with acetyl-CoA carboxylase-\(\alpha\) (ACCA), a rate-limiting enzyme of \textit{de novo} fatty acid synthesis. This association between AKR1B10 and ACCA proteins was further confirmed by co-immunoprecipitation with ACCA antibody and pulldown assays with recombinant AKR1B10 protein. Intracellular fluorescent studies showed that AKR1B10 and ACCA proteins co-localize in the cytoplasm of RAO-3 cells. More interestingly, small interfering RNA-mediated AKR1B10 knock down increased ACCA degradation through ubiquitination-proteasome pathway and resulted in >50% decrease of fatty acid synthesis in RAO-3 cells. These data suggest that AKR1B10 is a novel regulator of the biosynthesis of fatty acid, an essential component of the cell membrane, in breast cancer cells.

Aldo-keto reductase family 1 B10 (AKR1B10,\(^2\) also designated aldose reductase-like-1, ARL-1) is a novel protein identified from human hepatocellular carcinoma (1). This protein belongs to the ald-keto reductase superfamily, a group of proteins implicated in intracellular detoxification, cell carcinogenesis, and cancer therapeutics (2–5). AKR1B10 is primarily expressed in the colon and small intestine with low levels in the liver, thymus, prostate, and testis (1). However, this gene is overexpressed in 54% of human hepatocellular carcinoma, 84.4% of lung squamous cell carcinoma, and 29.2% of lung adenocarcinoma in smokers, making it a potential diagnostic and/or prognostic marker (1, 6, 7). AKR1B10 is an enzyme that efficiently catalyzes the reduction of carbonyls to corresponding alcohols with NADPH as a co-enzyme (1). Recent studies demonstrate that AKR1B10 expression facilitates growth of cancer cells, enhances their clonogenic capability, and reduces their susceptibility to reactive carbonyls such as acrolein and crotonaldehyde (8, 9). In vitro, AKR1B10 also shows strong enzymatic activity toward all-trans-retinal, 9-cis-retinal, and 13-cis-retinal, reducing them to the corresponding retinols. The diversity of retinal metabolism may diminish intracellular retinoic acid, a signaling molecule regulating cell proliferation and differentiation (4, 10).

The current study presents a novel biological function of AKR1B10, regulating long chain fatty acid synthesis, in human breast cancer cells. During tumorigenic transformation of human mammary epithelial cells (HMEC), AKR1B10 is up-regulated and associates with acetyl-CoA carboxylase-\(\alpha\) (ACCA). ACCA is a rate-limiting enzyme of \textit{de novo} synthesis of long chain fatty acids, catalyzing the formation of malonyl-CoA by ATP-dependent carboxylation of acetyl-CoA (11, 12). Increased lipogenesis is an important characteristic of cancer cells and likely contributes to the development and progression of cancer, but the regulatory mechanisms remain to be elucidated (13, 14). Up-regulation of lipogenic enzymes such as fatty acid synthase and ACCA has been documented in a variety of cancers, including breast, prostate, ovary, lung, colon, and endometrial cancers (11, 14–19). Long chain fatty acids are building blocks of membranes and precursors of lipid second messengers, playing a critical role in cell proliferation and division; ACCA knock-out mice are embryonically lethal (20, 21). In prostate and breast cancer cells, RNA interference-mediated silencing of ACCA inhibits fatty acid synthesis, arrests cell cycle, and induces caspase-mediated apoptosis (22–24). This study identifies AKR1B10 as a novel regulator of fatty acid \textit{de novo} synthesis, providing a new target for the manipulation of cancer cell growth.
Cell Culture and AKR1B10 Silencing by siRNA—RAO-1, RAO-2, and RAO-3 cells were cultured in DFCI-1 medium characterized previously (25). For AKR1B10 gene silencing, small-interfering RNAs (siRNA) were synthesized and introduced into RAO-3 cells as described previously (9).

Gel Filtration—30 ml of Sephacryl-S 300 (Amersham Biosciences) was packed into a column (10 × 300 mm) following the product instructions. RAO-3 cells (1 × 10^6) were lysed in a buffer (0.2% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, 150 mM NaCl, 2 mM EDTA, 50 mM Tris-HCl, pH 7.0, and 10% glycerol) (26). After centrifugation at 10,000 × g, 4 °C for 20 min, soluble proteins were loaded to the column at 4 °C. Proteins were eluted with 10 mM KCl in 20 mM Tris-HCl, pH 7.0, at 0.2 ml/min and collected at 0.5 ml/fraction. Ferritin (440 kDa), yeast alcohol dehydrogenase (150 kDa), bovine albumin (67 kDa), and carbonic anhydrase (29 kDa) were used as standards.

Anion Exchanger Chromatography—Total volume of 2 ml of DEAE-Sepharose (Amersham Biosciences) was packed into a column. RAO-3 cells were lysed in the buffer as described above. Soluble proteins were applied to the column connected to a fast protein liquid chromatography system (Bio-Rad) at 0.25 ml/min. Flow-through was collected. After being washed with 10 column volumes of lysis buffer, binding proteins were eluted with NaCl at a gradient of 10 to 1000 mM and collected at 0.5 ml/fraction.

Co-immunoprecipitation—RAO-3 cells were lysed as above. Soluble proteins (500 μg) were incubated with 5 μg of anti-AKR1B10 (refer to supplemental data for activity and specificity of AKR1B10 antibody produced in our laboratory) or 5 μg of anti-ACCA (Cell Signaling) antibodies at 4 °C overnight, followed by incubation with 40 μl of slurry-Sepharose protein A/G beads at 4 °C for 1 h with gentle shaking. Beads were collected by brief centrifugation and washed five times with lysis buffer (see above). Proteins were eluted with 50 μl of 100 mM glycine, pH 2.5, and separated on 8–12% SDS-PAGE, followed by Coomassie Blue staining or Western blot. Rabbit IgG (5 μg) was used as a negative control.

Mass Spectrometry Assay—Co-purified protein by immunoprecipitation was collected from SDS-PAGE. Gel slices were destained and digested in 25 μl of sequencing grade trypsin (12.5 ng/μl in 25 mM ammonium bicarbonate; G-Biosciences) at 37 °C overnight. The digested sample was dried using a Savant SpeedVac concentrator and resuspended in 13 nl of 13 buffer (0.2% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, 150 mM NaCl, 2 mM EDTA, 50 mM Tris-HCl, pH 7.0, and 10% glycerol) (26). After centrifugation at 10,000 × g, 4 °C for 20 min, soluble proteins were loaded to the column at 4 °C. Proteins were eluted using a linear gradient of water/acetonitrile containing 0.1% formic acid from water to acetonitrile in 60 min. Ferritin (440 kDa), yeast alcohol dehydrogenase (150 kDa), bovine albumin (67 kDa), and carbonic anhydrase (29 kDa) were used as standards.

FIGURE 1. Up-regulation of AKR1B10 and ACCA in RAO-3 cells. Soluble proteins (30 μg for AKR1B10 or 80 μg for ACCA) from RAO-1, RAO-2, and RAO-3 cells were separated on SDS-PAGE (12% for AKR1B10 or 8% for ACCA) and blotted on nitrocellulose membranes (Bio-Rad). Western blot was performed as described under Materials and Methods. β-Actin was used as a loading control. Refer to Results for RAO cells.

and PEAKS (Bioinformatics Solutions Inc. Waterloo, ON, Canada), and blasted against the NCBI NR data base.

Western Blot Analysis—Western blot was performed as previously described (8). ACCA antibody was used at 1:1000 in blocking buffer (Li-Cor Biosciences).

Recombinant AKR1B10 Protein Preparation and Pulldown Assay—Recombinant AKR1B10 protein with His tag was purified as described previously (8). (Refer to supplemental data for quality of protein.) To perform pulldown assay, 5 μg of recombinant AKR1B10 protein was incubated with 500 μg of soluble protein (cell lysates) at 4 °C for 2 h. Ni-NTA-agarose beads (40 μl) were then added and incubated at 4 °C for 1 h. After brief centrifugation, Ni-NTA-agarose beads were pelleted and washed with lysis buffer (see above) five times. Proteins were released by heating at 95 °C in 5 × SDS-PAGE loading buffer for 3 min and subjected to Western blot. A Ni-NTA-agarose bead control was run in parallel.

Transient Transfection and Fluorescent Localization—AKR1B10-EGFP fusion protein expression vector was transfected into RAO-3 cells as previously described (8). After culture for 48 h, cells were fixed in ice-cold methanol for 10 min and then in acetone for 1 min, followed by incubation with ACCA antibody (1:10) at 4 °C overnight. Texas Red-labeled secondary antibody (1:1000; GeneTex) was used to visualize ACCA antibody. Dual-color detection by confocal laser scan microscopy (TCS SP2 system; Leica, Bensheim, Germany) was performed for cellular distribution and localization of AKR1B10-EGFP and ACCA proteins.

Fatty Acid Synthesis—Fatty acid synthesis was determined by measuring radiolabeled acetic acid incorporation. In 24-well plates, cells were pulsed with 1 μCi/well of 13C-labeled acetic acid (Moravek) for 4 h. Cells were trypsinized and washed with phosphate-buffered saline three times. One fourth of the cell suspension was lysed for protein quantitation with protein assay dye (Bio-Rad). 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 supernatant was washed with 0.2 volume of dH2O. Aqueous phase and interface were removed, and organic phase was dried in speed
AKR1B10 Regulates Fatty Acid Synthesis

A) Gel filtration chromatography

B) Ion exchange chromatograph

FIGURE 2. AKR1B10 protein complexes in RAO-3 cells. A, gel filtration chromatography. RAO-3 cell lysates prepared as described under "Materials and Methods" were separated by a Sephacryl-S300 column, and fractions (0.5 ml each) were subjected to Western blot for AKR1B10 protein (lower panel). In the upper panel, solid triangles indicate molecular mass standards (ferritin, 440 kDa; yeast alcohol dehydrogenase, 150 kDa; bovine albumin, 67 kDa; carbonic anhydrase, 29 kDa). Solid circles represent density of AKR1B10 band in each fraction. B, ion exchange chromatography. RAO-3 cell lysates were applied to a DEAE-Sepharose column. Fractions (0.5 ml each) were subjected to Western blot. Reloading of flow-through onto gel filtration column showed that the AKR1B10 complex was eluted as the first peak of ~300 kDa (lower panel). Flow-T, flow-through.

A) Coomassie blue staining

B) Western blot

C) Ion exchange chromatography

FIGURE 3. A candidate protein associating with AKR1B10. Protein complex identified in Fig. 2 was immunoprecipitated using AKR1B10 antibody and separated on 8% SDS-PAGE as described under Materials and Methods. A, Coomassie Blue staining, indicating a candidate protein with ~250 kDa. M, molecular mass markers (kDa). B, Western blot. 5 μl of immunoprecipitated sample was subjected to Western blot analysis, confirming the presence of AKR1B10 protein. Lane 1, immunoprecipitation with AKR1B10 antibody; lane 2, rabbit IgG control. C, ion exchange chromatography of ACCA, performed as described in Fig. 2B. Fractions were pooled for Western blot analysis due to low amounts of ACCA protein. E2–5, fractions 2–5; E7–9, fractions 7–9; and E10–12, fractions 10–12.

Vacuum. Lipids were dissolved in 4.0 ml of liquid scintillation mixture, and radioactivity was measured by a scintillation counter (Beckman).

RESULTS

AKR1B10 and ACCA are Up-regulated in Tumorigenic HMEC Cells—In a previous study, we generated a panel of HMEC cell models (25). Primary HMEC cells were immortalized by a catalytic subunit of telomerase, generating RAO-1 cells. By introduction of activated H-RasQ61L into RAO-1 cells, RAO-2 and RAO-3 cells were isolated. RAO-2 cells with H-RasQ61L expression show transformational growth but are not tumorigenic in nude mice; RAO-3 cells possess an extra deletion of Rab25 gene besides H-RasQ61L expression and show anchorage-independent growth in soft agar and tumorigenesis in nude mice. In this study, we observed a tumorigenic-related up-regulation of both AKR1B10 and ACCA proteins in RAO-3 cells (Fig. 1).

Two Distinct AKR1B10 Protein Complexes in RAO-3 Cells—To explore the biological function of the induced AKR1B10 protein in the tumorigenic RAO-3 cells, we first examined its biochemical properties. As shown in Fig. 2A, gel filtration chromatography exhibited two AKR1B10 protein peaks with masses of ~40 and ~300 kDa, respectively. To verify this finding, anion exchange chromatography was conducted using DEAE-Sepharose ion exchange column. In this assay, AKR1B10 protein was present in two distinct portions. A portion of AKR1B10 remained in flow-through while the other bound to the column with elution at 200–300 mM NaCl (Fig. 2B, upper panel). Reloading the flow-through onto the gel filtration column revealed that AKR1B10 in flow-through represented the peak of ~300 kDa (Fig. 2B, lower panel). The AKR1B10 bound to ion exchange column stood for the peak at ~40 kDa (data not shown). These data indicate the existence of two distinct AKR1B10 forms in RAO-3 cells, monomers with calculated molecular mass of 36 kDa and complexes with a mass of ~300 kDa, that associate with other protein(s).

AKR1B10 Associates with ACCA in RAO-3 Cells—To identify proteins that associate with AKR1B10, immunoprecipitation was performed with a specific anti-AKR1B10 antibody generated in our laboratory. (Supplemental data demonstrate the activity and specificity of AKR1B10 antibody.) After separation on SDS-PAGE, Coomassie Blue staining indicated a candidate protein at ~250 kDa, which was not present in the rabbit IgG control (Fig. 3A).
AKR1B10 Regulates Fatty Acid Synthesis

AKR1B10 presence in precipitate was confirmed by Western blot (Fig. 3B). Protein mass spectrometry analysis identified that this candidate protein is ACCA with 265 kDa. Fig. 4 shows an example of mass spectrometry analysis of tryptic digests representing amino acids 244–266. Interestingly, association with AKR1B10 also altered ACCA binding to DEAE-Sepharose (Fig. 3C).

Association of AKR1B10 with ACCA was confirmed by co-immunoprecipitation using ACCA-specific antibody and AKR1B10 protein pulldown assay. In RAO-3 cells, AKR1B10 protein was specifically co-precipitated by ACCA antibody (Fig. 5A) and ACCA was pulled down by histidine-tagged recombinant AKR1B10 protein (Fig. 5B). (Refer to supplementary data for the quality of AKR1B10 recombinant protein.) In addition, intracellular fluorescent studies showed that ectopic AKR1B10-EGFP co-localizes with cellular ACCA protein in the cytoplasm of RAO-3 cells (Fig. 6).

AKR1B10 Affects Fatty Acid Synthesis in RAO-3 Cells by Regulating ACCA Degradation through Ubiquitination-proteasome Pathway—To understand the biological significance of this protein-protein association, we further examined the effect of AKR1B10 knock down on ACCA protein stability and de novo fatty acid synthesis. At first, we checked ACCA protein degradation pathways in RAO-3 cells. Fig. 7A shows that exposing RAO-3 cells to epoxomicin, a proteasome inhibitor, resulted in a dose-dependent accumulation of ACCA protein, indicating its degradation through ubiquitination-proteasome pathway as reported previously (27, 28). Thereafter, we down-regulated AKR1B10 protein in RAO-3 cells with chemically synthesized siRNA 1 and 2 characterized in our previous studies (9) and examined ACCA protein levels. As shown in Fig. 7B, AKR1B10 knock down caused a notable decrease of ACCA protein levels. This ACCA protein reduction was blocked by proteasome inhibitor epoxomicin, suggesting that by direct association AKR1B10 prevents ACCA degradation through ubiquitination-proteasome pathway. Furthermore, we examined the effect of AKR1B10 knock down on de novo fatty acid synthesis in RAO-3 cells by measuring the incorporation of 14C-labeled acetic acid. The results showed that AKR1B10 knock down resulted in >50% decrease of fatty acid synthesis (Fig. 7C). Taken together, these data suggest that AKR1B10 affects fatty acid synthesis via regulating ACCA stability.

DISCUSSION

Lipogenic alterations are early events of cancer development. In many types of cancers, lipogenic genes such as fatty acid synthase and ACCA are up-regulated (14–16). For instance, fatty acid synthase is overexpressed in the earliest stages of prostate neoplastic transformation (PIN lesions) and expression levels are positively related to the grades of PIN lesions and invasive carcinomas (16, 29). Recent studies have demonstrated that in tumor cells newly synthesized lipids are mainly phospholipids, the major components of cell membranes, meeting the need of rapid cell division. More importantly, newly synthesized lipids are enriched with saturated or monounsaturated fatty acids. Saturated fatty acids tend to partition into detergent-resistant membrane microdomains or rafts, which mediate cell migration, signal transduction, and intracellular trafficking (13, 14, 30, 31). In this study, we found that AKR1B10 is
FIGURE 7. Effect of AKR1B10 on ACCA stability and fatty acid synthesis in RAO-3 cells. A, ACCA accumulation by epoxomicin (Epo). Cells were treated by epoxomicin, a proteasome inhibitor, for 12 h, and lysates (50 µg for AKR1B10 or 100 µg for ACCA) were subjected to Western blot as described under Materials and Methods. Epoxomicin induced a dose-dependent accumulation of ACCA protein. B, ACCA reduction induced by AKR1B10 silencing. RAO-3 cells were transiently transfected by two siRNAs as described in text and then exposed or not to epoxomicin at 200 nM for 12 h. Cell lysates were subjected to Western blot. AKR1B10 knock down induced ACCA decrease that was blocked by epoxomicin. Control (Ctrl), scrambled siRNA; R1, siRNA 1; R2, siRNA 2. C, fatty acid synthesis. RAO-3 cells transfected by siRNA 1 and 2 were incubated in 24-well plates for 72 h to trigger AKR1B10 knockdown and then pulsed with 1 µCi/well of 13C-labeled acetic acid for 4 h. Lipids were extracted as described under Materials and Methods, and acetic acid incorporation was determined by radioactivity (cpm/µg protein). Data represent the mean ± S.D. from three independent experiments. *, p < 0.01 compared with scrambled siRNA control.

A) ACCA accumulation by epoxomicin

B) AKR1B10 silencing and ACCA stability

C) Fatty acid synthesis

HMEC cells, affects fatty acid synthesis by regulating the stability of ACCA protein and thus becomes a novel target for the modulation of de novo fatty acid synthesis in cancer cells.

REFERENCES

1. Cao, D., Fan, S. T., and Chung, S. S. (1998) J. Biol. Chem. 273, 11429–11435
2. Jin, J., Krishack, P. A., and Cao, D. (2006) Front Biosci. 11, 2767–2773
3. Hyndman, D., Bauman, D. R., Heredia, V. V., and Penning, T. M. (2003) Chem. Biol. Interact 143–144, 621–631
4. Crosas, B., Hyndman, D. J., Gallego, O., Martiras, S., Pares, X., Flynn, T. G., and Farres, J. (2003) Biochim. Biophys. Acta 1687–1690
5. Lee, K. W., Ko, B. C., Jiang, Z., Cao, D., and Chung, S. S. (2001) Anti-Cancer Drugs 12, 129–132
6. Penning, T. M. (2005) Clin. Cancer Res. 11, 1687–1690
7. Fukumoto, S., Yamauchi, N., Moriguchi, H., Hippo, Y., Watanabe, A., Shibahara, J., Taniguchi, H., Ishikawa, S., Ito, H., Yamamoto, S., Iwami, H., Hironaka, M., Ishikawa, Y., Niki, T., Sobar, Y., Kodama, T., Nishimura, M., Fukayama, M., Dosaka-Akita, H., and Aburatani, H. (2005) Clin. Cancer Res. 11, 1776–1785
8. Yu, X., Yan, R., Robbins, S., Krishack, P. A., Liao, D. F., and Cao, D. (2007) Toxicol. Sci. 97, 562–568
9. Yan, R., Xu, X., Ma, J., Liu, Z., Adeyanju, M., and Cao, D. (2007) Int. J. Cancer 121, 2301–2306
10. Dragunov, K. H., Rigas, J. R., and Dmitrovsky, E. (2000) Oncologist 5, 361–368
11. Witters, L. A., Widmer, J., King, A. N., Fasshi, K., and Kuhajda, F. (1994) Int. J. Biochem. 26, 589–594
12. Zang, Y., Wang, T., Xie, W., Wang-Fischer, Y. L., Getty, L., Han, J., Corkey, B. E., and Guo, W. (2005) Obes. Res. 13, 1530–1539
13. Rouquette-Jazdanian, A. K., Pelassy, C., Breittmayer, J. P., Cousin, J. L., and Aussel, C. (2002) Biochim. Biophys. Acta 1563, Pt. 3, 645–655
14. Swinnen, V., Heemers, H., van de Sande, T., de Schriijver, E., Brussel-
mans, K., Heyns, W., and Verhoeven, G. (2004) J. Steroid Biochem. Mol. Biol. 92, 273–279
15. Kuhajda, F. P. (2000) Nutrition 16, 202–208
16. Swinnen, J. V., Roskams, T., Joniau, S., Van Poppel, H., Oyen, R., Baert, L., Heyns, W., and Verhoeven, G. (2002) Int. J. Cancer 98, 19–22
17. Rossi, S., Graner, E., Febo, P., Weinstein, L., Bhattacharya, N., Onody, T., Bubley, G., Balk, S., and Loda, M. (2003) Mol. Cancer Res. 1, 707–715
18. Milgraum, L. Z., Witters, L. A., Pasternack, G. R., and Kuhajda, F. P. (1997) Clin. Cancer Res. 3, 2115–2120
19. Yahagi, N., Shimano, H., Hasegawa, K., Ohashi, K., Matsuzaka, T., Najima, Y., Sekiya, M., Tomita, S., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Nagai, R., Ishibashi, S., Kadowaki, M., Makuuchi, M., Ohashi, K., Ohnishi, S., Osuga, J., and Yamada, N. (2005) Eur. J. Cancer 41, 1316–1322
20. Swinnen, J. V., Brusselmans, K., and Verhoeven, G. (2006) Curr. Opin. Clin. Nutr. Metab. Care 9, 358–365
21. Abu-Elheiga, L., Matzuk, M. M., Kordari, P., Oh, W., Shaikenov, T., Gu, Z., and Wakil, S. J. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 12011–12016
22. Chajes, V., Cambot, M., Moreau, K., Lenoir, G. M., and Joulin, V. (2006) Cancer Res. 66, 5287–5294
23. Brusselmans, K., De Schrijver, E., Verhoeven, G., and Swinnen, J. V. (2005) Cancer Res. 65, 6719–6725
24. Tong, L., and Harwood, H. J., Jr. (2006) J. Cell. Biochem. 99, 1476–1488
25. Cheng, J. M., Ding, M., Aribi, A., Shah, P., and Rao, K. (2006) Int. J. Cancer 118, 2957–2964
26. Moreau, K., Dizin, E., Ray, H., Luquain, C., Lefai, E., Foulelle, F., Billaud, M., Lenoir, G. M., and Venezia, N. D. (2006) J. Biol. Chem. 281, 3172–3181
27. Qi, L., Heredia, J. E., Altarejos, J. Y., Screaton, R., Goebel, N., Niessen, S., Macleod, I. X., Liew, C. W., Kulkarni, R. N., Bain, J., Newgard, C., Nelson, M., Evans, R. M., Yates, J., and Montminy, M. (2006) Science 312, 1763–1766
28. Neels, J. G., and Olefsky, J. M. (2006) Science 312, 1756–1758
29. Shurbaji, M. S., Kalbfleisch, J. H., and Thurmond, T. S. (1996) Hum. Pathol. 27, 917–921
30. Simons, K., and Toomre, D. (2000) Nat. Rev. Mol. Cell. Biol. 1, 31–39
31. Manes, S., Mira, E., Gomez-Mouton, C., Lacalle, R. A., Keller, P., Labrador, J. P., and Martinez, A. C. (1999) EMBO J. 18, 6211–6220
32. Brownsey, R. W., Boone, A. N., Elliott, J. E., Kulp, J. E., and Lee, W. M. (2006) Biochem. Soc. Trans. 34, Pt. 2, 223–227
33. Fediu, S., Gaidhu, M. P., and Ceddia, R. B. (2006) J. Lipid Res. 47, 412–420
34. Dyck, J. R., Kudo, N., Barr, A. J., Davies, S. P., Hardie, D. G., and Lopaschuk, G. D. (1999) Eur. J. Biochem. 262, 184–190
35. Magnard, C., Bacherier, R., Vincent, A., Jaquinod, M., Kieffer, S., Lenoir, G. M., and Venezia, N. D. (2002) Oncogene 21, 6729–6739