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Acetyl-CoA carboxylase rewires cancer metabolism to allow cancer cells to survive inhibition of the Warburg effect by cetuximab

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Abstract

Cetuximab inhibits HIF-1-regulated glycolysis in cancer cells, thereby reversing the Warburg effect and leading to inhibition of cancer cell metabolism. AMP-activated protein kinase (AMPK) is activated after cetuximab treatment, and a sustained AMPK activity is a mechanism contributing to cetuximab resistance. Here, we investigated how acetyl-CoA carboxylase (ACC), a downstream target of AMPK, rewires cancer metabolism in response to cetuximab treatment. We found that introduction of experimental ACC mutants lacking the AMPK phosphorylation sites (ACC1_S79A and ACC2_S212A) into head and neck squamous cell carcinoma (HNSCC) cells protected HNSCC cells from cetuximab-induced growth inhibition. HNSCC cells with acquired cetuximab resistance contained not only high levels of T172-phosphorylated AMPK and S79-phosphorylated ACC1 but also an increased level of total ACC. These findings were corroborated in tumor specimens of HNSCC patients treated with cetuximab. Cetuximab plus TOFA (an allosteric inhibitor of ACC) achieved remarkable growth inhibition of cetuximab-resistant HNSCC xenografts. Our data suggest a novel paradigm in which cetuximab-mediated activation of AMPK and subsequent phosphorylation and inhibition of ACC is followed by a compensatory increase in total ACC, which rewires cancer metabolism from glycolysis-dependent to lipogenesis-dependent.

Keywords

Warburg effect; ACC; AMPK; HIF-1; EGFR; Cetuximab

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CONFLICTS OF INTEREST

None.

1. Introduction

The Warburg effect, also known as “aerobic glycolysis”, refers to a phenomenon first observed by Otto Warburg over 80 years ago in which cancer cells use glycolysis to generate lactate as the primary means for glucose metabolism, even when the cellular level of oxygen is sufficient for oxidation of pyruvate [1]. It is believed that cancer cells, by consuming large amounts of glucose via glycolysis, gain sufficient biomass-building materials for cell growth and proliferation. Targeting the Warburg effect, therefore, has been considered an attractive approach for cancer treatment [2-5]. We previously reported that cetuximab, a Food and Drug Administration-approved anti-epidermal growth factor receptor (EGFR) antibody, exerts its antitumor activity at least in part via inhibiting the Warburg effect through downregulating hypoxia-inducible factor-1 alpha (HIF-1 α) [6-8], the regulatory subunit of HIF-1, which is a key transcription factor that regulates almost every biochemical step of glycolysis, as well as glucose uptake and lactate production and excretion [9,10].

More recently, we reported that inhibition of HIF-1 transcriptional activity by cetuximab does not always lead to successful inhibition of cell proliferation [11]. In human head and neck squamous cell carcinoma (HNSCC) cells, we observed that the response to cetuximab-mediated growth inhibition was linked to the activity status of the cell energy sensor AMP-activated protein kinase (AMPK). HNSCC cells with a low basal level of AMPK activity were more sensitive to cetuximab-induced growth inhibition and exhibited a transient activation of AMPK after cetuximab treatment. In contrast, HNSCC cells with a high basal level of AMPK activity were less sensitive to cetuximab-induced growth inhibition despite effective inhibition of EGFR downstream signaling by cetuximab [11].

An emerging paradigm is that cancer cells may rewire metabolic pathways from a glycolysis-dependent pattern to a lipogenesis-dependent pattern with fatty acid oxidation in response to treatments targeting the Warburg effect [12]. AMPK, through phosphorylation of acetyl-CoA carboxylase (ACC), plays an important role in maintaining cell energy homeostasis when cells are under stress [13-15]. AMPK-mediated phosphorylation of ACC1 at Ser79 [16] and ACC2 at Ser221 (Ser212 in mice) [17] is a well-described mechanism that leads to inhibition of fatty acid synthesis and stimulation of fatty acid β -oxidation, through which cells survive under energy stress. However, in vivo data supporting this paradigm, particularly data from patients, have been limited. Few studies have used clinical data to investigate the impact of the AMPK and ACC axis on cancer cell response to therapies targeting the Warburg effect.

In this study, by using ACC1 and ACC2 experimental mutants lacking the corresponding AMPK phosphorylation sites (ACC1_S79A and ACC2_S212A) [18], we further dissected the role of ACC in HNSCC cell response to cetuximab treatment. We first examined the role of the ACC mutants in an experimental Warburg effect model in which overexpression of HIF-1 α in HEK293 cells renders the cells highly dependent on glucose supply in culture medium. We found that both ACC1 activity and ACC2 activity are indispensable for HEK293 cell survival in low glucose culture, which mimics the outcome of therapies targeting the Warburg effect. We next demonstrated that ACC rewires cancer metabolism to

allow HNSCC cells to survive inhibition of the Warburg effect by cetuximab. We showed that co-targeting ACC with TOFA, an allosteric inhibitor of ACC, substantially improved the response of cetuximab-resistant HNSCC xenografts to cetuximab treatment. We further corroborated our observations in tumor specimens from patients with HNSCC treated with or without cetuximab.

2. Materials and methods

2.1 Patients

Tumor specimens were obtained from patients treated at the Department of Head and Neck Surgical Oncology, Tianjin Medical University Cancer Institute & Hospital, Tianjin, China, during 2007-2013. Tumor specimens from six patients who underwent post-cetuximab surgery and had complete medical records available were used for immunohistochemical evaluation of T172-phosphorylated AMPK, S79-phosphorylated ACC1, and total ACC. Surgical specimens from another 12 patients with complete medical records who were treated with the same chemotherapy regimen without cetuximab during the same period were used as controls. Informed consent was obtained for research use of these specimens.

2.2 Cell culture

293 human kidney embryonic cells (HEK293) and human HNSCC cells (HN5, FaDu, Tu159, OSC19, MDA1986, UMSCC1, and Tu167) were maintained in high glucose (4.5 g/L) Dulbecco's modified Eagle's medium/F12 medium (50/50, v/v) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 g/mL streptomycin in a 5% CO₂ atmosphere at 37°C as previously described [19,20].

2.3 Plasmids

The complementary DNAs encoding HIF-1α_P402A/P564A and HIF-1α_ ODD, originally provided by Dr. L. Eric Huang (University of Utah School of Medicine), were subcloned into a modified pLEX-MCS lentiviral vector (Thermo Fisher Scientific) via the *Bam*HI and *Not*I sites. ACC1_S79A and ACC2_S212A pLent6-D-TOPO vectors were provided by Dr. Nissim Hay (University of Illinois at Chicago).

2.4 Western blotting

The procedure for Western blotting was previously described [8,11]. The primary antibodies used for Western blotting and their sources were as follows: HIF-1α and SREBP-1c, BD Biosciences; T172-phosphorylated AMPK, AMPK, S79-phosphorylated ACC1, ACC1, ACC2, and FASN, Cell Signaling Technology; and β-actin, Sigma-Aldrich.

2.5 Live/dead cell viability assay and cell survival proportions

The LIVE/DEAD cell viability assay kit (Life Technologies) was used to distinguish live versus dead cells as we recently described [11,21]. For determination of the cell survival proportions after culture in low glucose medium (1 mM) supplemented with 0.5% FBS, cells were plated at a low density ($0.7-1 \times 10^5$ cells per well in a 12-well plate) in regular medium; the next day, the culture medium was switched to low glucose medium. At various

time points after the switch to low glucose medium, the cells were washed once with PBS and incubated with 4 μ M calcein acetoxymethyl ester. Five different areas were then randomly selected and imaged under a fluorescence microscope. The imaging data were analyzed using the ImageJ software program, and cell survival proportions were calculated by dividing the number of surviving cells at various time points after the switch to low glucose medium by the number of surviving cells before the switch [22-26].

2.6 Glucose consumption assay

Cells were seeded in six-well plates at 5×10^5 cells/well in 3 mL of phenol red-free 5 mM glucose, 0.5% FBS cell culture medium as described above. At various time points after treatment, an aliquot of 50 μ L of the conditioned medium was collected from each well and diluted with 950 μ L of distilled water (1:20). The glucose concentration in the diluted medium was measured using the Glucose (GO) assay kit (Sigma-Aldrich) as we previously described [8].

2.7 Apoptosis assays

Apoptosis was measured by detection of PARP cleavage using Western blotting with an antibody that recognizes both cleaved and uncleaved PARP (Cell Signaling Technology); by quantitative measurement of the levels of cytoplasmic histone-associated DNA fragments (mononucleosomes and oligonucleosomes) using a Cell Death Detection ELISA kit (Roche Diagnostics Corp.); or by quantitative measurement of apoptotic cells using a flow cytometer after staining of cells with FITC-conjugated annexin V and propidium iodide (Life Technologies), according to the vendors' protocols [7,21,27].

2.8 Knockdown of ACC1 and ACC2 gene expression by siRNA

ACC1-targeted siRNA (target DNA sequence #1, CUAUGAGGGAGUCAAGUAU; #2, CUAUGAGGUGGAUCGGAGA), ACC2-targeted siRNA (#1, CCUACAAUGGGAACAGCUA; #2, GAACUUAACCGGAUGCGUA), and control siRNA were purchased from Sigma-Aldrich. The siRNA-mediated gene expression knockdown was performed as we previously described [8,11].

2.9 Quantitative real-time PCR

Total RNA was extracted from cells using a modified chloroform/phenol procedure (Trizol; Life Technologies). First-strand cDNA was generated using the High Capacity cDNA Reverse Transcription Kit (Invitrogen). The fluorescent real-time PCR reaction was performed in a thermal cycler (7500 Fast Real-Time PCR System, Applied Biosystems) for 40 cycles (denaturation at 95°C for 15 sec and annealing at 60°C for 30 sec) in iQ SYBR green supermix (Bio-Rad) with a final volume of 10 μ L containing 0.5 μ L of cDNA template with the specific primers targeting human ACC1 (ACACA) (forward: 5'-ATGTCTGGCTTGACCTAGTA-3'; reverse: 5'-CCCCAAAGCGAGTAACAAATTCT-3') or ACC2 (ACACB) (forward: 5'-AGAAGACAAGAAGCAGGCAAAC-3'; reverse: 5'-GTAGACTCACGAGATGAGCCA-3') or 0.5 μ L of an internal control cDNA with specific primers targeting human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward: 5'-CATGTTTCGTCATGGGGTGAACCA-3', reverse: 5'-

AGTGATGGCATGGACTGTGGTCAT-3'). Fluorescent readings from real-time PCR reaction products were quantitatively analyzed by determining the difference in cycle number of crossing point (CP) between the target gene (ACC1 and ACC2) and GAPDH. Relative gene expression was calculated using the Pfaffl method [28], and changes of ACC1 and ACC2 mRNA expression in cetuximab-treated cells were obtained by comparison with the ACC1 and ACC2 mRNA expression in untreated cells.

2.10 Cell survival and proliferation assays

Cell survival and proliferation assays were performed using the MTT (methylthiazolyldiphenyl-tetrazolium bromide) method as we previously described [29,30]. The relative number of surviving cells in each group was determined by measuring the optical density (OD) of the cell lysates at an absorbance wavelength of 570 nm. The OD value in each treatment group was then expressed as a percentage of the OD value in the untreated control cells, and the percentage values were plotted against treatments.

2.11 Animal studies and bioluminescence tumor imaging

Animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at The University of Texas MD Anderson Cancer Center and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Swiss female nude mice (5-6 weeks old) were obtained from the colony facility maintained by the Department of Experimental Radiation Oncology at MD Anderson Cancer Center. HN5-R and UMSCC1 cells infected with a pLEX-based recombinant lentivirus containing a firefly luciferase cDNA were implanted subcutaneously on the right flanks of nude mice (6×10^6 cells/mouse in 100 μ L of serum-free medium). Treatments were started on day 15, when xenograft volume reached approximately 120 mm³. TOFA was first dissolved in DMF (10 mg/mL) and then diluted 1:50 with PBS. Tumor volume was calculated using the formula $\pi/6 \times ab^2$ (a: length; b: width, $a > b$). Bioluminescent imaging of xenografts was performed with the Xenogen in vitro imaging system in living animals after intraperitoneal injection of D-luciferin (3.3 mg/100 μ L) and induction of anesthesia by inhalation of 2.5% isoflurane (IsoSol; Vedco, Inc., St. Joseph, MO). Bioluminescent imaging data were analyzed using Living Image 4.3.1 software.

2.12 Immunohistochemistry analysis

Immunohistochemical staining was performed using the VECTASTAIN ABC kit (Vector Laboratories). Briefly, after deparaffinization and rehydration, 5- μ m sections were subjected to heat-induced epitope retrieval using a microwave oven for 10 min in 0.01 M citrate retrieval buffer (pH 6.0). Endogenous peroxidase activity was blocked for 10 min in 3% hydrogen peroxide. Nonspecific binding was blocked with 1% goat serum for 1 h at room temperature. Slides were then incubated with primary antibody at 4°C overnight. The following primary antibodies were used: T172-phosphorylated AMPK (1:100, Cell Signaling), S79-phosphorylated ACC1 (1:100, Cell Signaling), and total ACC (1:100, Cell Signaling). Slides were subjected to color development with 3,3'-diaminobenzidine and were counterstained with hematoxylin. A negative control slide without primary antibody was always included for each staining.

2.13 Statistical analyses

Each experiment was repeated two times or more. A two-tailed unpaired Student t test was used to compare two groups of independent samples.

3. Results

3.1 ACC plays a critical role in maintaining cell survival in low glucose culture of cells exhibiting an increased dependence on glucose metabolism

Cancer cells are more dependent on glucose metabolism than normal cells because of the Warburg effect. To investigate the role of ACC in cancer cell response to treatment targeting the Warburg effect, we first established a Warburg effect model by overexpressing HIF-1 α , a key regulator of the Warburg effect, in HEK293 cells. We chose nonmalignant HEK293 cells for establishing the Warburg effect model because this allowed us to investigate the role of ACC in cell response to therapy targeting the Warburg effect in the absence of oncogenic mutations or mutational inactivation of tumor suppressor genes, which are common in cancer cells and could complicate interpretation of our results.

Because wild-type HIF-1 α is highly unstable in normoxic culture, we used two degradation-resistant (constitutively active) HIF-1 α mutants, HIF-1 α _P402A/P564A and HIF-1 α _ODD. Experimental mutation of these two hydroxylable proline residues (P402 and P564) on HIF-1 α or deletion of the entire oxygen-dependent degradation domain (ODD) containing the two proline sites results in a high level of HIF-1 α due to stabilization of HIF-1 α in normoxia [31,32].

As we expected, overexpression of either HIF-1 α mutant led to a significant increase in glucose consumption in the transfected HEK293 cells due to increased glycolysis driven by HIF-1 (Fig. 1A, B). HEK293 cells overexpressing either HIF-1 α mutant were significantly more sensitive to low glucose culture (1 mM, i.e., 0.18 g/L), which mimics the outcome of therapies targeting the Warburg effect, than were control vector-infected HEK293 cells (Fig. 1C). As a result of HIF-1 α overexpression/stabilization-induced energy stress in low glucose culture, the energy sensor AMPK was activated (shown by increased T172 phosphorylation of AMPK in Fig. 1A). The activated AMPK phosphorylated ACC (shown by increased S79 phosphorylation of ACC1 in Fig. 1A) and inhibited ACC function, thereby switching cell metabolism from the ATP-consuming process of fatty acid synthesis to the ATP-generating process of fatty acid oxidation to maintain cell survival.

However, expression of ACC1_S79A or ACC2_S212A, each of which lacks the corresponding AMPK phosphorylation site [18], particularly expression of ACC1_S79A, strongly protected the survival of HEK293 HIF-1 α _P402A/P564A cells in low glucose culture (Fig. 1D–F). Overexpression of ACC1_S79A or ACC2_S212A had no impact on low glucose culture-induced activation of AMPK or inhibition of endogenous ACC in HEK293 HIF-1 α _P402A/P564A cells (Fig. 1D). Taken together, these findings indicated that ACC enzyme activity helps maintain cell survival. To further support this conclusion, we subjected HEK293 and HEK293 HIF-1 α _P402A/P564A cells to treatment with ACC1-specific or ACC2-specific siRNA and found that knockdown of endogenous ACC1 or ACC2, particularly ACC1, amplified the effect of low glucose culture for both types of cells

(**Fig. 1G–I**). Only 20% of HEK293 HIF-1 α _P402A/P564A cells remained alive 16 h after knockdown of ACC1 (**Fig. 1I**), compared to approximately 70% of parental HEK293 cells with ACC1 knockdown (**Fig. 1H**).

Together, these data indicate that although ACC (ACC1 and ACC2) is inhibited in response to HIF-1 α overexpression/stabilization-induced energy stress in low glucose culture, ACC, particularly ACC1, also helps maintain cell survival under these same conditions.

3.2 High levels of phosphorylated AMPK, phosphorylated ACC1, and total ACC correlate with resistance of HNSCC cells to cetuximab

We next sought to corroborate this paradigm that ACC helps maintain cell survival under energy stress in cancer cells. Our earlier work showed that cetuximab activated AMPK while it inhibited proliferation of HNSCC cells, even when cultured in high glucose medium (25 mM, i.e., 4.5 g/L), via downregulation of HIF-1 α and inhibition of HIF-1-regulated glycolysis [6-8,11,33]. Here, in a panel of HNSCC cell lines, we found an inverse correlation between basal levels of T172-phosphorylated (activated) AMPK and S79-phosphorylated (inhibited) ACC1 and cellular sensitivity to cetuximab (**Fig. 2A**). This correlation was confirmed in two isogenic pairs of cells with acquired resistance to cetuximab following chronic exposure to cetuximab: HN5/HN5-R and FaDu/FaDu-R [8,11]. The levels of T172-phosphorylated AMPK and S79-phosphorylated ACC1 were stably elevated in the resistant sublines (HN5-R and FaDu-R) (**Fig. 2B**). Furthermore, we found that overexpression of the constitutively active HIF-1 α -P402A/P564A mutant in HN5 cells potentiated cetuximab-induced AMPK activation and ACC inhibition, and conferred significant resistance to cetuximab (**Fig. 2C**), similar to the findings in the HN5-R and FaDu-R sublines (**Fig. 2B**).

An important finding was that, compared to the cetuximab-sensitive cells, the cetuximab-resistant cells not only had a higher level of S79-phosphorylated ACC1 (as shown in **Fig. 2B**) but also had a higher level of total ACC (**Fig. 3A, left**). This increase in total ACC was not due to increased transcription, as indicated by our findings that the mRNA levels of both ACC1 and ACC2, measured by real-time PCR, differed minimally between HN5 and HN5-R cells and were modestly lowered by cetuximab in HN5 cells but not in HN5-R cells (**Fig. 3A, right**) and that the level of SREBP-1c, a key transcription factor for activating ACC expression, was similar between HN5 and HN5-R cells and was decreased by cetuximab in HN5 cells but not in HN5-R cells (**Fig. 3A, left**). Results similar to those in HN5-R cells were observed in UMSCC1 and MDA1986 cells (**Fig. 3B**), two other HNSCC cell lines that are naturally resistant to cetuximab-induced growth inhibition compared to the effect in cetuximab-sensitive HNSCC cell lines (**Fig. 2A**). Interestingly, however, ACC was remarkably more stable in HN5-R cells than in HN5 cells (**Fig. 3C**), suggesting that a post-translational mechanism was responsible for the increase in total ACC in cetuximab-resistant cells. This increase in total ACC was likely to compensate for the functional inhibition of ACC by AMPK.

Together, these findings suggest that an increased level of total ACC, particularly ACC1, is linked to resistance of HNSCC cells to treatment with cetuximab, which targets the Warburg effect [8].

3.3 Combining cetuximab with TOFA enhances response of cetuximab-resistant HNSCC cells to cetuximab in vitro and in vivo

We next found that expression of ACC1_S79A or ACC2_212A in HN5 cells, particularly expression of ACC1_S79A, strongly protected the cells against cetuximab-induced inhibition of cell growth and survival (**Fig. 4A**). In contrast, the cetuximab-resistant HN5-R cells already contain a high level of ACC; therefore, transfection of ACC1_S79A or ACC2_S212A had no noticeable further effect on promoting cetuximab resistance (**Fig. 4B**). Knockdown of endogenous ACC1 or ACC2 by respective siRNAs did not enhance the level of apoptosis, measured by the level of increase in DNA fragmentation, after cetuximab treatment in parental HN5 cells (**Fig. 4C**), but knockdown of endogenous ACC1 or ACC2, particularly knockdown of ACC1, significantly enhanced the level of apoptosis induced after cetuximab treatment in HN5-R cells (**Fig. 4D**). Similar results were observed in UMSCC1 cells (**Supplementary Fig. 1**).

Consistent with the results following knockdown of ACC expression, HN5-R cells were remarkably more sensitive than HN5 cells to treatment with TOFA (5-[tetradecyloxy]-2-furoic acid), a cell-permeable allosteric inhibitor of ACC [34-36] (**Fig. 5A**). UMSCC1 and MDA1986, also demonstrated high sensitivity to TOFA (**Fig. 5A**). Moreover, chronic exposure of UMSCC1 cells to TOFA in culture resulted in a TOFA-resistant subline, UMSCC1-TofaR, that was more sensitive to cetuximab than UMSCC1 (**Fig. 5B-D**). Together, these data showed that ACC1 plays a critical role in the resistance of HNSCC cells to cetuximab treatment.

We next tested our hypothesis that co-targeting ACC is a rational strategy for treating cetuximab-resistant cancer cells. As shown in **Figure 6A-D** and in **Supplementary Figures 2 and 3**, TOFA plus cetuximab markedly increased apoptosis induction in HN5-R, UMSCC1, and MDA1986 cells compared to no treatment or either single treatment. In nude mice bearing HN5-R xenografts, three of eight mice responded to TOFA alone and three of eight mice responded to cetuximab alone, including one mouse that had complete disappearance of tumor, but TOFA plus cetuximab produced a significantly greater therapeutic effect (eight of eight mice responded to combination treatment), as shown by two-dimensional tumor measurements (**Fig. 6E**) and by IVIS tumor imaging (**Fig. 6F**). TOFA was well tolerated: there was no noticeable loss of body weight of the mice treated with TOFA or TOFA plus cetuximab (**Fig. 6E**). Immunohistochemical evaluation of the tumor specimens showed higher levels of T172-phosphorylated AMPK in cetuximab-treated HN5-R xenografts than in control HN5-R xenografts (**Fig. 6G**). TOFA and TOFA plus cetuximab increased the level of T172-phosphorylated AMPK and downregulated the level of total ACC in HN5-R xenografts (**Fig. 6G**), consistent with the findings from cell culture (**Fig. 6A**). However, in contrast with the findings from cell culture, the level of S79-phosphorylated ACC1 was also reduced in HN5-R xenografts treated with TOFA or TOFA plus cetuximab, likely because of the decrease in the level of total ACC. The therapeutic

strategy of combining TOFA with cetuximab was repeated using UMSSC1 xenografts, and results showed that the outcome of TOFA plus cetuximab was dependent on the TOFA dose (**Supplementary Fig. 4**).

3.4 Levels of phosphorylated AMPK, phosphorylated ACC, and total ACC are higher in tumor specimens from cetuximab-treated HNSCC patients than from cetuximab-untreated HNSCC patients

Under current National Comprehensive Cancer Network guidelines, cetuximab is approved for patients with metastatic HNSCC after failure of standard chemotherapy [37]. Few patients with HNSCC who undergo a standard 8-week cetuximab treatment undergo surgery afterwards. However, we were able to collect tumor specimens from six patients who underwent post-cetuximab surgery, including one who underwent surgery both before and after the treatment regimen containing cetuximab (**Supplementary Fig. 5**). Tumor specimens from another 12 patients who received the same chemotherapy regimen without cetuximab and underwent post-chemotherapy surgery served as a control group. Clinicopathological characteristics of these patients and results of immunohistochemical evaluation of the levels of T172-phosphorylated AMPK, S79-phosphorylated ACC1, and total ACC in the surgical specimens are summarized in **Fig. 7** (left). In the patient who underwent surgery both before and after cetuximab treatment (patient 6), comparison of the surgical samples showed markedly higher levels of these markers after cetuximab treatment (**Fig. 7**, right).

Although the number of tumor specimens that we examined was limited, our findings clearly indicate that levels of T172-phosphorylated AMPK, S79-phosphorylated ACC, and total ACC were increased after cetuximab treatment. Taken together with the findings from cell culture and animal studies, these data suggest the model depicted in **Fig. 8**. Our results support the conclusion that ACC plays an important role in rewiring cancer metabolism to help cancer cells survive therapy targeting the Warburg effect.

4. Discussion

In this study, by using ACC mutants lacking the AMPK phosphorylation sites (ACC1_S79A and ACC2_S212A), we demonstrated that the activity of ACC plays an important role in helping cells to survive therapies targeting the Warburg effect. We reached this conclusion by performing experiments using both an experimental model of the Warburg effect regulated by HIF-1 and HNSCC cell models following cetuximab treatment, which targets the Warburg effect through downregulation of HIF-1 α [8]. Importantly, the conclusion is supported by the data from our animal studies and our analysis of tumor specimens from patients treated with cetuximab.

We recently reported that transient activation of cell energy sensor AMPK is an early marker of cellular response to cetuximab and that high and sustained AMPK activity is an important mechanism by which cancer cells survive cetuximab treatment [11]. Activation of AMPK leads to phosphorylation and inhibition of ACC, through which the ATP-consuming process of fatty acid synthesis is inhibited and the ATP-generating process of fatty acid oxidation is stimulated, and through which cells may survive energy stress. We present new evidence, in

the current study, indicating that high levels of T172-phosphorylated (activated) AMPK, S79-phosphorylated (inhibited) ACC, and total ACC correlated with resistance to cetuximab in a panel of HNSCC cell lines. We showed that cetuximab plus the ACC inhibitor TOFA had remarkable antitumor activity against cetuximab-resistant HNSCC xenografts and, more importantly, that the levels of T172-phosphorylated AMPK, S79-phosphorylated ACC and total ACC were significantly higher in tumor specimens of cetuximab-treated patients than in tumor specimens of corresponding control patients.

Both ACC1 and ACC2 can generate malonyl-CoA but at different subcellular locations [15]. Malonyl-CoA serves as the substrate for fatty acid synthesis that occurs in the cytoplasm, and functions as the regulator of fatty acid oxidation that occurs in the mitochondria [15]. Both processes are highly regulated and play important roles in the metabolism of fatty acids. While AMPK-mediated reversible inhibition of ACC1 leads to a decline in the level of malonyl-CoA and a resultant decline in the synthesis of fatty acids [15,38], AMPK-mediated reversible inhibition of ACC2 leads to a decline in the level of malonyl-CoA and a resultant activation of carnitine palmitoyltransferase-1 (CPT-1), which promotes fatty acid oxidation in mitochondria. In our studies, we found that the protective effect of ACC1_S79A against glucose deprivation or cetuximab treatment was consistently stronger than that of ACC2_S212A. Conversely, knockdown of endogenous ACC1 consistently produced a stronger effect than knockdown of endogenous ACC2 on enhancing glucose deprivation-induced or cetuximab-induced inhibition of cell survival. Because the malonyl-CoA generated by ACC1 and ACC2 is segregated into different subcellular locations [15], our findings suggest a scenario wherein fatty acid synthesis could increase without a concomitant decrease in fatty acid oxidation. Therefore, a future research direction could be to determine whether inhibition of fatty acid oxidation alone, for example, inhibition of CPT-1, has any impact on the response of cetuximab-resistant HNSCC cells to cetuximab treatment.

In the current study, we found that a post-translational mechanism was most likely responsible for an increase in the level of total ACC in cetuximab-resistant HNSCC cells. This increase in the level of total ACC likely is a compensatory mechanism of the cells in response to AMPK activation-induced inhibition of ACC function. However, the detailed biochemical mechanism underlying the increased stability of ACC remains unknown. A separate study is clearly warranted.

In conclusion, our studies indicate that upregulation of ACC, mainly ACC1, in response to AMPK activation-induced inhibition of ACC allows cancer cells to survive therapy with cetuximab, which targets the Warburg effect by downregulating HIF-1 α [8,11]. Many molecularly targeted anti-cancer drugs that have been approved or are in development, including inhibitors of EGFR, PI3K, and mTOR and even some chemotherapeutic agents, like cisplatin [39], downregulate HIF-1 α directly or indirectly [9,40,41], which can lead to activation of AMPK. Thus, co-targeting ACC, which we found is a rational therapeutic strategy for treatment of cetuximab-resistant HNSCC, could also improve the outcomes of other therapies targeting the Warburg effect.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Warburg O. On the origin of cancer cells. *Science*. 1956; 123:309–314. [PubMed: 13298683]
2. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009; 324:1029–1033. [PubMed: 19460998]
3. Vander Heiden MG. Targeting cancer metabolism: a therapeutic window opens. *Nat. Rev. Drug Discov*. 2011; 10:671–684. [PubMed: 21878982]
4. Tennant DA, Duran RV, Gottlieb E. Targeting metabolic transformation for cancer therapy. *Nat. Rev. Cancer*. 2010; 10:267–277. [PubMed: 20300106]
5. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
6. Luwor RB, Lu Y, Li X, Mendelsohn J, Fan Z. The antiepidermal growth factor receptor monoclonal antibody cetuximab/C225 reduces hypoxia-inducible factor-1 alpha, leading to transcriptional inhibition of vascular endothelial growth factor expression. *Oncogene*. 2005; 24:4433–4441. [PubMed: 15806152]
7. Li X, Lu Y, Liang K, Pan T, Mendelsohn J, Fan Z. Requirement of hypoxia-inducible factor-1alpha down-regulation in mediating the antitumor activity of the anti-epidermal growth factor receptor monoclonal antibody cetuximab. *Mol. Cancer Ther*. 2008; 7:1207–1217. [PubMed: 18483308]
8. Lu H, Li X, Luo Z, Liu J, Fan Z. Cetuximab reverses the Warburg effect by inhibiting HIF-1-regulated LDH-A. *Mol. Cancer Ther*. 2013; 12:2187–2199. [PubMed: 23920275]
9. Semenza GL. HIF-1: upstream and downstream of cancer metabolism. *Curr. Opin. Genet. Dev*. 2010; 20:51–56. [PubMed: 19942427]
10. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene*. 2010; 29:625–634. [PubMed: 19946328]
11. Li X, Lu Y, Lu H, Luo J, Hong Y, Fan Z. AMPK-mediated energy homeostasis and associated metabolic effects on cancer cell response and resistance to cetuximab. *Oncotarget*. 2015; 6:11507–11518. [PubMed: 25871473]
12. Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr. Cellular fatty acid metabolism and cancer. *Cell Metab*. 2013; 18:153–161. [PubMed: 23791484]
13. Hardie DG. The AMP-activated protein kinase pathway--new players upstream and downstream. *J. Cell Sci*. 2004; 117:5479–5487. [PubMed: 15509864]
14. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol*. 2012; 13:251–262. [PubMed: 22436748]
15. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. *J. Lipid Res*. 2009; 50(Suppl):S138–S143. [PubMed: 19047759]
16. Ha J, Daniel S, Broyles SS, Kim KH. Critical phosphorylation sites for acetyl-CoA carboxylase activity. *J. Biol. Chem*. 1994; 269:22162–22168. [PubMed: 7915280]
17. Winder WW, Wilson HA, Hardie DG, Rasmussen BB, Hutber CA, Call GB, Clayton RD, Conley LM, Yoon S, Zhou B. Phosphorylation of rat muscle acetyl-CoA carboxylase by AMP-activated protein kinase and protein kinase. A, J. *Appl. Physiol* (1985.). 1997; 82:219–225. [PubMed: 9029219]

18. Jeon SM, Chandel NS, Hay N. AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. *Nature*. 2012; 485:661–665. [PubMed: 22660331]
19. Lu H, Liang K, Lu Y, Fan Z. The anti-EGFR antibody cetuximab sensitizes human head and neck squamous cell carcinoma cells to radiation in part through inhibiting radiation-induced upregulation of HIF-1 α . *Cancer Lett*. 2012; 322:78–85. [PubMed: 22348829]
20. Zhao M, Sano D, Pickering CR, Jasser SA, Henderson YC, Clayman GL, Sturgis EM, Ow TJ, Lotan R, Carey TE, Sacks PG, Grandis JR, Sidransky D, Heldin NE, Myers JN. Assembly and initial characterization of a panel of 85 genomically validated cell lines from diverse head and neck tumor sites. *Clin. Cancer Res*. 2011; 17:7248–7264. [PubMed: 21868764]
21. Lu Y, Shi C, Qiu S, Fan Z. Identification and validation of COX-2 as a co-target for overcoming cetuximab resistance in colorectal cancer cells. *Oncotarget*. 2016 in press.
22. Drey LL, Graber MC, Bieschke J. Counting unstained, confluent cells by modified bright-field microscopy. *Biotechniques*. 2013; 55:28–33. [PubMed: 23834382]
23. Jensen EC. Quantitative analysis of histological staining and fluorescence using ImageJ. *Anat. Rec. (Hoboken.)*. 2013; 296:378–381. [PubMed: 23382140]
24. Gavet O, Pines J. Progressive activation of CyclinB1-Cdk1 coordinates entry to mitosis. *Dev. Cell*. 2010; 18:533–543. [PubMed: 20412769]
25. Burgess A, Vigneron S, Brioudes E, Labbe JC, Lorca T, Castro A. Loss of human Greatwall results in G2 arrest and multiple mitotic defects due to deregulation of the cyclin B-Cdc2/PP2A balance. *Proc. Natl. Acad. Sci. U. S. A*. 2010; 107:12564–12569. [PubMed: 20538976]
26. McCloy RA, Rogers S, Caldon CE, Lorca T, Castro A, Burgess A. Partial inhibition of Cdk1 in G 2 phase overrides the SAC and decouples mitotic events. *Cell Cycle*. 2014; 13:1400–1412. [PubMed: 24626186]
27. Lu Y, Li X, Lu H, Fan Z. 1, 9-Pyrazoloanthrones downregulate HIF-1 α and sensitize cancer cells to cetuximab-mediated anti-EGFR therapy. *PLoS One*. 2010; 5:e15823. [PubMed: 21209911]
28. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res*. 2001; 29:e45. [PubMed: 11328886]
29. Liang K, Qiu S, Lu Y, Fan Z. Autocrine/paracrine erythropoietin regulates migration and invasion potential and the stemness of human breast cancer cells. *Cancer Biol. Ther*. 2014; 15:89–98. [PubMed: 24100272]
30. Liang K, Esteva FJ, Albarracin C, Stemke-Hale K, Lu Y, Bianchini G, Yang CY, Li Y, Li X, Chen CT, Mills GB, Hortobagyi GN, Mendelsohn J, Hung MC, Fan Z. Recombinant human erythropoietin antagonizes trastuzumab treatment of breast cancer cells via Jak2-mediated Src activation and PTEN inactivation. *Cancer Cell*. 2010; 18:423–435. [PubMed: 21075308]
31. Kageyama Y, Koshiji M, To KK, Tian YM, Ratcliffe PJ, Huang LE. Leu-574 of human HIF-1 α is a molecular determinant of prolyl hydroxylation. *FASEB J*. 2004; 18:1028–1030. [PubMed: 15084514]
32. Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. HIF-1 α induces cell cycle arrest by functionally counteracting Myc. *EMBO J*. 2004; 23:1949–1956. [PubMed: 15071503]
33. Li X, Fan Z. The epidermal growth factor receptor antibody cetuximab induces autophagy in cancer cells by downregulating HIF-1 α and Bcl-2 and activating the Beclin 1/hVps34 complex. *Cancer Res*. 2010; 70:5942–5952. [PubMed: 20634405]
34. McCune SA, Harris RA. Mechanism responsible for 5-(tetradecyloxy)-2-furoic acid inhibition of hepatic lipogenesis. *J. Biol. Chem*. 1979; 254:10095–10101. [PubMed: 39932]
35. Halvorson DL, McCune SA. Inhibition of fatty acid synthesis in isolated adipocytes by 5-(tetradecyloxy)-2-furoic acid. *Lipids*. 1984; 19:851–856. [PubMed: 6549204]
36. Wang C, Xu C, Sun M, Luo D, Liao DF, Cao D. Acetyl-CoA carboxylase- α inhibitor TOFA induces human cancer cell apoptosis. *Biochem. Biophys. Res. Commun*. 2009; 385:302–306. [PubMed: 19450551]
37. Brana I, Siu LL. Locally advanced head and neck squamous cell cancer: treatment choice based on risk factors and optimizing drug prescription. *Ann. Oncol*. 2012; 23(Suppl 10):x178–x185. [PubMed: 22987958]

38. Rasmussen BB, Holmback UC, Volpi E, Morio-Liondore B, Paddon-Jones D, Wolfe RR. Malonyl coenzyme A and the regulation of functional carnitine palmitoyltransferase-1 activity and fat oxidation in human skeletal muscle. *J. Clin. Invest.* 2002; 110:1687–1693. [PubMed: 12464674]
39. Ai Z, Lu Y, Qiu S, Fan Z. Overcoming cisplatin resistance of ovarian cancer cells by targeting HIF-1-regulated cancer metabolism. *Cancer Lett.* 2016; 373:36–44. [PubMed: 26801746]
40. Semenza G. Signal transduction to hypoxia-inducible factor 1. *Biochem. Pharmacol.* 2002; 64:993–998. [PubMed: 12213597]
41. Onnis B, Rapisarda A, Melillo G. Development of HIF-1 inhibitors for cancer therapy. *J Cell Mol. Med.* 2009; 13:2780–2786. [PubMed: 19674190]

Highlights

- ACC plays a critical role in maintaining cell survival in low glucose conditions
- A post-cetuximab increase in total ACC contributes to cetuximab resistance
- ACC rewires cancer metabolism from glycolysis-dependent to lipogenesis-dependent
- Cetuximab plus ACC inhibitor enhances response of cetuximab-resistant HNSCC cells

Figure 1.

ACC plays a critical role in maintaining survival of cells exhibiting an increased dependence on glucose in low glucose culture. **A–C**, HEK293 cells infected with a recombinant lentivirus as indicated were subjected to Western blotting using the antibodies indicated (**A**). Conditioned media from culture of the cells in normal glucose medium (5 mM) for the indicated time periods were used for comparing glucose consumption (**B**). Cell survival proportions after culture in low glucose medium (1 mM) were determined as described in Materials and Methods (**C**). **D–F**, HEK293 cells co-infected with lentivirus containing HIF-1 α _P402A/P564A or not and lentivirus containing ACC1_S79A or ACC2_S212A as indicated were subjected to Western blotting using the antibodies indicated (**D**) and to analysis of cell survival proportions (**E**, **F**). **G–I**, HEK293 cells infected with lentivirus containing HIF-1 α _P402A/P564A or not and transfected with a control siRNA, ACC1 siRNA, or ACC2 siRNA as described in Materials and Methods were subjected to Western blotting using the antibodies indicated (**G**) and to analysis of cell survival proportions (**H**, **I**). All differences between control and experimental conditions are statistically significant except those marked “ns” (not significant).

Figure 2.

High levels of phosphorylated AMPK, phosphorylated ACC1, and total ACC correlate with resistance of HNSCC cells to cetuximab. **A** and **B**, The indicated HNSCC cell lines and sublines were subjected to MTT assays following treatment with cetuximab (20 nM) or not for 72 h and to Western blotting using the antibodies indicated following treatment with cetuximab (20 nM) or not for 24 h. **C**, HN5 cells infected with a control lentivirus or the virus containing HIF-1α_P402A/P564A were subjected to MTT assays following treatment

with cetuximab (20 nM) or not for 72 h and to Western blotting using the antibodies indicated following treatment with cetuximab (20 nM) or not for 24 h.

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Figure 3.

HNSCC cells with low sensitivity to cetuximab contain a high basal level of ACC associated with increased stability of ACC. **A** and **B**, Cetuximab-sensitive (HN5) and cetuximab-resistant (HN5-R, UMSCC1, and MDA1986) cells were treated with cetuximab (20 nM) or not for 24 h. Cell protein lysates were subjected to Western blotting using the antibodies indicated (left panels). RNA samples were used to determine the levels of ACC1 and ACC2 mRNA in the cells with and without cetuximab treatment by real-time PCR as described in Materials and Methods (right panels). **C**, HN5 and HN5-R cells were cultured in the

presence of the protein biosynthesis inhibitor cycloheximide (CHX, 10 μ M) for the indicated times. Cell protein lysates were collected at each indicated time point and subjected to Western blotting using the antibodies indicated. Densitometric values of the ACC protein band at each time point were plotted as shown.

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Figure 4.

Knockdown of ACC sensitized cetuximab-resistant cells to cetuximab treatment. **A** and **B**, HN5 (**A**) and HN5-R (**B**) cells infected with lentivirus containing ACC1_S79A or ACC2_S212A as indicated were subjected to MTT assays following treatment with cetuximab (20 nM) or not for 72 h and to Western blotting using the antibodies indicated following treatment with cetuximab (20 nM) or not for 24 h. **C** and **D**, HN5 cells (**C**) and HN5-R cells (**D**) were transfected with a control siRNA, one of two different ACC1 siRNAs, or one of two different ACC2 siRNAs as indicated for 48 h and then treated with cetuximab (20 nM) or not for an additional 24 h. Cell lysates were then subjected to Western blotting using the antibodies indicated and to analysis of apoptosis measured by fold increases in DNA fragmentation as described in Materials and Methods. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0005$. ns: not significant.

Figure 5.

Cetuximab-resistant HNSCC cells are more sensitive to TOFA treatment than are cetuximab-sensitive HNSCC cells. **A**, Cetuximab-sensitive (HN5) and cetuximab-resistant (HN5-R, UMSCC1, and MDA1986) cells were treated with the indicated concentrations of TOFA or vehicle (DMSO) for 3 days and then subjected to MTT assays. **B** and **C**, UMSCC1 and UMSCC1-TofaR cells (a subline of UMSCC1 cells produced by chronic exposure to TOFA for over 3 months at concentrations gradually increased from 0.1 to 5 μM) were treated with cetuximab (20 nM) or not for 24 h. The cell lysates were then subjected to Western blotting using the antibodies indicated (B) and to apoptosis ELISA for levels of DNA fragmentation (C). **D**, UMSCC1 and UMSCC1-TofaR cells were treated with cetuximab (20 nM) or not for 24 h and then subjected to a live/dead cell viability assay and observed under a fluorescence microscope (scale bars, 200 μm). Shown to the right of the photos are the numbers of live and dead cells after the treatments determined by using ImageJ software. Only dead cells in each group were compared. * $p < 0.05$; *** $p < 0.0005$; ns, not significant.

Figure 6.

Combining cetuximab with TOFA enhances response of cetuximab-resistant HNSCC cells to cetuximab in vitro and in vivo. **A–D**, HN5 and HN5-R cells were treated with cetuximab (20 nM), TOFA (0.3 μ M), or the combination for 24 h and subjected to Western blotting using the antibodies indicated (**A**), to apoptosis ELISA for levels of DNA fragmentation (**B**), and to flow cytometry analysis for detecting annexin-V-positive cells (**C**) or were treated for 6 days and then subjected to MTT assays (**D**). * p 0.05; ** p 0.01; *** p 0.0005. ns: not significant. **E–G**, HN5-R xenografts were treated intraperitoneally with cetuximab (50 μ g/mouse, twice a week), TOFA (1 mg/kg body weight, once daily, 5 days a week), or the combination for 4 weeks. The mice were weighed and the tumors were measured in two dimensions using digital calipers twice a week (mean $\pm$ SE) (**E**). At the end of treatment, the mice were subjected to IVIS imaging (**F**), and the tumor specimens were subjected to immunohistochemical staining using the antibodies indicated (**G**).

Clinicopathological characteristics and treatments of 18 patients with HNSCC

Case	Sex	Age yr	Location of SCC	Stage	Grade	Treatments
1	F	75	Buccal mucosa	IVA (T3N2M0)	G2	TPF+C+S
2	F	53	Buccal mucosa	IVA (T2N2M0)	G1	TPF+C+S
3	M	61	Tongue	IVA (T2N2M0)	G2	TPF+C+S→TPF
4	M	47	Floor of mouth	IVA (T2N2M0)	G3	TPF+C+S
5	F	67	Retromolar trigone	IVA (T3N2M0)	G3	TPF+C+S
6	F	56	Retromolar trigone	III (T2N1M0)→ IVA (T2N2M0)	G1→G3	TPF+S→TPF+C+S
7	F	68	Tongue	IVA (T2N2M0)	G1	TPF+S
8	M	49	Retromolar trigone	IVA (T3N2M0)	G2	TPF+S
9	F	33	Tongue	III (T3N0M0)	G2	TPF+S
10	F	62	Retromolar trigone	III (T3N0M0)	G3	TPF+S
11	M	50	Base of tongue	IVA (T4aN2M0)	G2-3	TPF+S
12	F	82	Upper alveolar ridge	IVA (T3N2M0)	G2-3	TPF+S
13	M	66	Floor of mouth	III (T3N1M0)	G2-3	TPF+S
14	M	55	Tongue	IVA (T3N2M0)	G2	TPF+S
15	M	57	Base of tongue	IVA (T3N2M0)	G2-3	TPF+S
16	M	62	Retromolar trigone	IVA (T4aN2M0)	G2-3	TPF+S
17	M	62	Base of tongue	IVA (T4aN2M0)	G3	TPF+S
18	F	58	Retromolar trigone	IVA (T3N2M0)	G2-3	TPF+S

Levels of AMPK-T172p, ACC-S79p, and total ACC by IHC in HNSCC tumors from patients with and without cetuximab

Expression level	No cetuximab (n=12)	Cetuximab (n=6)	p value
AMPK-T172p			
Negative or low	9	0	0.003
High	3	6	
ACC-S79p			
Negative or low	7	1	0.09
High	5	5	
ACC			
Negative or low	10	0	0.001
High	2	6

Figure 7. Levels of phosphorylated AMPK, phosphorylated ACC, and total ACC are higher in tumor specimens from cetuximab-treated HNSCC patients than in tumor specimens from cetuximab-untreated HNSCC patients. Shown at left are a summary of patients' clinicopathological characteristics and treatments received (top) and a summary of results of immunohistochemical evaluation of the levels of phosphorylated AMPK, phosphorylated ACC, and total ACC in patients' surgical specimens (bottom). F, female; M, male; SCC, squamous cell carcinoma; TPF, docetaxel + cisplatin + 5-fluorouracil; →, recurrence; C, cetuximab; S, surgery; IHC, immunohistochemical analysis; AMPK-172p, T172-phosphorylated AMPK; ACC-S79p, S79-phosphorylated ACC1. Shown at right are photos with low and high magnifications of the results of immunohistochemical staining for the

indicated markers in tumor specimens obtained from patient 6 before and after cetuximab treatment.

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Figure 8.

Proposed model of the mechanism underlying the effects of combination treatment with cetuximab and TOFA. Blue arrows illustrate the directions of flow of the metabolic pathways. Red symbols indicate the effects of cetuximab on the pathways (↑, increase; ↓ decrease; ⊥, inhibition), whereas green symbols (↓ or ⊥) indicate the effects of TOFA. The instance of “ACC” set in a large font represents a compensatory increase in total ACC level due to AMPK-mediated phosphorylation and inhibition of ACC (ACC-p).