Compartmentalized activities of the pyruvate dehydrogenase complex sustain lipogenesis in prostate cancer

Jingjing Chen1,2, Ilaria Guccini1, Diletta Di Mitri1, Daniela Brina1, Ajinkya Revandkar1,2, Manuela Sarti3, Emiliano Pasquini3, Abdullah Alajati1, Sandra Pinton1, Marco Losa1, Gianluca Civenni1, Carlo V. Catapano1, Jacopo Sgrignani1, Andrea Cavalli1, Rocco D’Antuono4, John M. Asara5, Andrea Morandi6, Paola Chiarugi6, Sara Crotti7, Marco Agostini7,8, Monica Montopoli9, Ionica Masgras10, Andrea Rasola10, Ramon García-Escudero11,12,13, Nicolas Delaleu14, Andrea Rinaldi1, Francesco Bertoni1, Johann de Bono15, Arkaitz Carracedo16,17,18 and Andrea Alimonti1,2,19*

The mechanisms by which mitochondrial metabolism supports cancer anabolism remain unclear. Here, we found that genetic and pharmacological inactivation of pyruvate dehydrogenase A1 (PDHA1), a subunit of the pyruvate dehydrogenase complex (PDC), inhibits prostate cancer development in mouse and human xenograft tumor models by affecting lipid biosynthesis. Mechanistically, we show that in prostate cancer, PDC localizes in both the mitochondria and the nucleus. Whereas nuclear PDC controls the expression of sterol regulatory element-binding transcription factor (SREBP)-target genes by mediating histone acetylation, mitochondrial PDC provides cytosolic citrate for lipid synthesis in a coordinated manner, thereby sustaining anabolism. Additionally, we found that PDHA1 and the PDC activator pyruvate dehydrogenase phosphatase 1 (PDP1) are frequently amplified and overexpressed at both the gene and protein levels in prostate tumors. Together, these findings demonstrate that both mitochondrial and nuclear PDC sustain prostate tumorigenesis by controlling lipid biosynthesis, thus suggesting this complex as a potential target for cancer therapy.

Mitochondrial metabolism is a source of energy and metabolic intermediates that serve various roles in processes including redox homeostasis, anabolism and epigenetics. There is an emerging association between upregulated mitochondrial metabolism and cancer pathogenesis and progression, but the molecular mechanisms underlying this association remain unknown. PDC is a gatekeeper multiprotein complex that catalyzes the conversion of pyruvate to acetyl coenzyme A (acetyl CoA), thereby regulating mitochondrial activity. This complex includes a major component, PDHA1, that can be dephosphorylated by the pyruvate dehydrogenase phosphatases (PDPs) Pdp1 and Pdp2 and phosphorylated by the pyruvate dehydrogenase kinases (Pdks). Whereas dephosphorylation of PDHA1 activates PDC, phosphorylation blocks its activity.

Results

Pdha1 knockout induces tumor suppression in mice and in human prostate tumors. We postulated that by impairing the functions of PDHA1 and PDC, we could restrict mitochondrial metabolism and be able to determine the consequent effects on prostate cancer biology. We thus inactivated Pdha1, in prostate-specific conditional Pten-null (Pten+/-) mice, which develop high-grade intraepithelial prostate tumors at an early age and invasive prostate cancer at a late age. The recombination of Pten exons 4 and 5 and Pdha1 exon 8 in Pten+/-; Pdha1+/- prostate in the presence of Cre recombinase was verified by genotyping (Supplementary Fig. 1a). Notably, Pten+/- mice developed tumors characterized by higher mRNA and/or protein levels of Pdha1, Dlat, Dld and Pdp1, but not Pdp2 or Pdks, and higher PDC activity.

The mechanisms by which mitochondrial metabolism supports cancer anabolism remain unclear. Here, we found that genetic and pharmacological inactivation of pyruvate dehydrogenase A1 (PDHA1), a subunit of the pyruvate dehydrogenase complex (PDC), inhibits prostate cancer development in mouse and human xenograft tumor models by affecting lipid biosynthesis. Mechanistically, we show that in prostate cancer, PDC localizes in both the mitochondria and the nucleus. Whereas nuclear PDC controls the expression of sterol regulatory element-binding transcription factor (SREBP)-target genes by mediating histone acetylation, mitochondrial PDC provides cytosolic citrate for lipid synthesis in a coordinated manner, thereby sustaining anabolism. Additionally, we found that PDHA1 and the PDC activator pyruvate dehydrogenase phosphatase 1 (PDP1) are frequently amplified and overexpressed at both the gene and protein levels in prostate tumors. Together, these findings demonstrate that both mitochondrial and nuclear PDC sustain prostate tumorigenesis by controlling lipid biosynthesis, thus suggesting this complex as a potential target for cancer therapy.
Fig. 1 | *Pdha1* knockout induces tumor suppression in mice and in human prostate tumors. **a.** Western blot analysis of the indicated proteins in wild type (WT), *Pdha1*^−/−*, *Pten*^−/−* and *Pten*^−/−*; *Pdha1*^−/−* prostates and tumors (*n* = 3 independent prostate samples). Uncropped images are in Supplementary Fig. 12. **b.** Top, PDC activity measurement in wild-type, *Pdha1*^−/−*, *Pten*^−/−* and *Pten*^−/−*; *Pdha1*^−/−* prostates and tumors (*n* = 3 independent prostate samples). Bottom, quantification of the indicated proteins normalized to wild-type levels in the prostate tumors in **a** (*n* = 3 independent prostate samples). **c.** Comparison of anterior prostate lobe volumes (mm^3*, two independent lobes per animal presented) from wild-type, *Pdha1*^−/−*, *Pten*^−/−* and *Pten*^−/−*; *Pdha1*^−/−* prostates and tumors from male mice of the indicated ages (*n*, number of mice). Inset, representative images of anterior prostate lobes. **d.** Representative micrographs in histopathological analysis (hematoxylin–eosin (H&E) staining and indicated proteins) of anterior prostates in *Pdha1*^−/−*, *Pten*^−/−* and *Pten*^−/−*; *Pdha1*^−/−* prostates and tumors from 12-week-old male mice (*n* = 3). Scale bar, 50 μm; insets are shown at higher magnification. Data for all the genotypes and images at lower magnification are shown in Supplementary Fig. 1c. **e, f.** Quantification of the percentage of Ki-67-positive cells (e) and invasive prostate glands (f) in prostates from mice of the indicated genotypes and ages (*n*, number of mice). **g.** Relative cell-number quantification by crystal violet staining in the indicated prostate cancer cell lines infected with shPDHA1 or scrambled control. Data are normalized to shRNA control. Inset, PDHA1 inhibition, validated by western blot analysis using two different shPDHA1 constructs. Uncropped images are in Supplementary Fig. 12 (*n* = 3 independent cell cultures). OD, optical density. **h.** Spheroid formation assays in LNCaP, 22Rv1 and PC3 cells infected with doxycycline-induced Tripz-shPDHA1 or scrambled control (*n* = 3 independent cell cultures). Scale bar, 50 μm. **i, j.** Quantification of spheroid diameter (i) and spheroid number per 500 cells (j) in LNCaP, 22Rv1 and PC3 cells infected with doxycycline-induced Tripz-shPDHA1 or scrambled Tripz-shRNA control (*n* = 3 independent cell cultures). **k, l.** Evaluation of tumor formation in xenotransplantation experiments of 22Rv1 (**k**) and PC3 (**l**) cells infected with the indicated shPDHA1 or scrambled-shRNA control. Inset, western blot validation of knockdown of PDHA1 in 22Rv1 and PC3 xenograft tumors. Uncropped images are in Supplementary Fig. 12 (*n* = 6 animals; 12 independent tumor samples). Error bars, s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. P values were determined by ANOVA with Bonferroni correction, unless otherwise noted.
Fig. 2 | Pdha1 inactivation induces tumor suppression by downregulating lipogenic genes. a, Gene expression profile analysis based on metabolic-pathway datasets (GOCC, Gene Ontology Cellular Component; KEGG, Kyoto Encyclopedia of Genes and Genomes; GOBO, Gene Expression-based Outcome; GOMF, Gene Ontology Molecular Function; HumanCyc; Reactome) in Ptenpten−/−; Pdha1pten−/− tumors versus Ptenpten−/− tumors. Dotted line indicates P = 0.05 (n = 3 independent prostate samples). FDR, false discovery rate. b, Western blot analysis of the indicated proteins in wild-type, Pdha1pten−/−, Ptenpten−/− and Pdha1pten−/−; Pdha1pten−/− prostates and tumors. Uncropped images are in Supplementary Fig. 12 (n = 3 independent cell cultures). c, Western blot analysis of the indicated proteins in 22Rv1 cells infected with doxycycline-induced Tripz-shRNA or scrambled control and treated with 100 µM acetate or vehicle over a 6-d period. Uncropped images are in Supplementary Fig. 12 (n = 3 independent cell cultures). d, Left, GSEA of SREBF-target genes in Ptenpten−/−; Pdha1pten−/− versus Ptenpten−/− prostate tumors. Normalized enriched scores (NES) are presented. Right, qRT–PCR analysis of the indicated SREBFs and target genes and genes in acetyl CoA compensatory pathways in mouse prostate and tumors of the indicated genotypes (n = 3 independent cell cultures). Data are mean ± s.d. e, qRT–PCR analysis of mRNA expression of the indicated SREBFs and target genes and genes in acetyl CoA compensatory pathways in 22Rv1 cells infected with shPDHA1 or scrambled shRNA control (n = 3 independent cell cultures). f, Quantitative RT–PCR analysis of ACLY and SQLE in 22Rv1 cells infected with doxycycline-induced Tripz-shPDHA1 or scrambled control and treated with acetate (100 µM) or vehicle for 6 d (n = 3 independent cell cultures). g, ChIP analysis showing the binding of SREBF1 and H3K9ac on the promoters of ACLY (g) and SQLE (h) in 22Rv1 and PC3 cells infected with doxycycline-induced Tripz-shPDHA1 or scrambled control and treated with 100 µM acetate or vehicle over a 6-d period (n = 3 independent cell cultures). h–k, Relative cell-number quantification by crystal violet staining of Pten−/− and Pten−/−; Pdha1−/− MEFs (i) and human 22Rv1 and PC3 cancer cells infected with doxycycline-induced Tripz-shPDHA1 or scrambled control (j and k) and treated with acetate (100 µM) or vehicle over a 6-d period (n = 3 independent cell cultures). Error bars, s.e.m. unless otherwise noted. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. P values were determined by ANOVA with Bonferroni correction, unless otherwise noted.
than that in normal prostates (Fig. 1a,b, Supplementary Fig. 1b and uncropped blots in Supplementary Fig. 12). Inactivation of Pdha1 in Pten\textsuperscript{pc−/−} tumors abrogated PDC activity and induced a strong growth inhibition in the prostate glands of mice of different ages (Fig. 1c). This effect was associated with a strong decrease in cell proliferation, as shown by decreases in Ki-67 staining and the number of glands affected by invasive prostate cancer (Fig. 1d–f and Supplementary Fig. 1c). The strong arrest in proliferation in Pten\textsuperscript{pc−/−}; Pdha1\textsuperscript{pc−/−} tumors and Pten\textsuperscript{−/−}; Pdha1\textsuperscript{−/−} mouse embryonic fibroblasts (MEFs) occurred independently of senescence\textsuperscript{19} and apoptosis, both of which occurred to a lesser extent than that in controls (Supplementary Fig. 1d–i). Notably, Pdha1 inactivation induced growth arrest exclusively in Pten\textsuperscript{−/−} MEFs, without altering the growth of wild-type MEFs (Supplementary Fig. 1g). Moreover, Pdha1 deletion in Pten-null tumors and MEFs did not affect the levels of phospho-Akt at Ser473 and the total amount of mitochondria, as indicated by the protein levels of voltage-dependent anion channel 1 (Vdac1)\textsuperscript{21} (Fig. 1a, Supplementary Fig. 1j) and uncropped blots in Supplementary Fig. 13).

Next, we assessed the status of both PDHA1 and PDP1 in human prostate cancers. Bioinformatic analysis showed that both PDHA1 and PDP1 are frequently amplified at the gene level and overexpressed in human prostate tumors. Interestingly, PDP1 was found to be amplified (3/3 available datasets) and overexpressed (9/11 datasets) in primary prostate tumors compared with normal prostate tissues, whereas PDHA1 was amplified (2/3 datasets) and overexpressed (7/13 datasets) (Supplementary Fig. 2a–e) in metastatic tumors compared with primary tumors. We also stained for PDHA1, phospho-PDHA1 and PDP1 on a tissue microarray (TMA) consisting of 128 prostate carcinoma and normal prostate samples. Immunohistochemistry analyses showed that both PDHA1 and PDP1 were frequently overexpressed in human prostate tumors, and their expression was higher in tumors with high Gleason scores (Supplementary Fig. 3a–e). In contrast, phospho-PDHA1 staining was high in only a small fraction of cases, which had low Gleason scores (Supplementary Fig. 3a,d). Interestingly, most cases with high PDP1 or PDHA1 staining were negative for phospho-PDHA1 (Supplementary Fig. 3e). Three out of four prostate cancer cell lines exhibited higher PDC activity than did nontransformed PNT2C2 prostate cells (Supplementary Fig. 3f). This result was associated with elevated protein levels of both PDHA1 and PDP1 and enhanced mitochondrial oxygen consumption dependent on both glucose and glutamine utilization (Supplementary Fig. 3f–i and uncropped blots in Supplementary Fig. 14). Together, these data demonstrated that in prostate tumors and in cancer cell lines, PDC is active.

We next inactivated PDHA1 in prostate cancer cells by using different PDHA1 short hairpin RNAs (shRNAs). In agreement with the results from the mouse model, we found that PDC inactivation resulted in an apoptosis-independent inhibition of cellular proliferation (Fig. 1g, Supplementary Fig. 3j,k and uncropped blots in Supplementary Fig. 12). We also detected lower mitochondrial respiration in cells infected with PDHA1 shRNA (shPDHA1) than in control cells (Supplementary Fig. 3l). The sphere-forming ability in LNCaP, 22Rv1 and PC3 cells was also impaired after PDHA1 inactivation (Fig. 1h–j, Supplementary Fig. 3m and uncropped blots in Supplementary Fig. 14). 22Rv1 and PC3 prostate cancer cells infected with shPDHA1 also formed smaller tumors in vivo than did controls (Fig. 1k,l and uncropped blots in Supplementary Fig. 12).

Pdha1 inactivation decreases mitochondrial intermediates. Next, we performed metabolomic analysis in wild-type prostates, Pdha1\textsuperscript{pc−/−} prostates, Pten\textsuperscript{pc−/−} prostates and Pten\textsuperscript{pc−/−}; Pdha1\textsuperscript{pc−/−} prostates. Pten\textsuperscript{pc−/−} tumors showed signs of upregulation of the tricarboxylic acid (TCA) cycle, as measured by elevated levels of citrate, α-ketoglutarate, acetyl CoA, ATP production and nicotinamide adenine dinucleotide (NADH) (Supplementary Fig. 4a–d, Supplementary Table 4 and Supplementary Note). Elevated extracellular lactate levels in Pten-null MEFs also suggested greater glycolytic flux in those cells than in controls (Supplementary Fig. 4e and Supplementary Note). Pdha1 inactivation in Pten-null tumors and MEFs resulted in lower levels of TCA-cycle intermediates such as citrate and α-ketoglutarate and affected the NADH/NAD\textsuperscript{+} ratio and ATP and acetyl CoA levels (Supplementary Fig. 4a–f, Supplementary Table 4 and Supplementary Note). However, glycolysis and lactate production were not consistently affected (Supplementary Fig. 4ae), in agreement with previous findings in different models\textsuperscript{16,22,23}. Of note, the oxaaoacetate levels were significantly higher in double-mutant mice, in line with a block in the cycle due to loss of acetyl CoA production downstream of PDC (Supplementary Fig. 4a,d and Supplementary Table 4). To determine whether Pdha1 inactivation induced changes in central carbon metabolism, we measured stationary flux\textsuperscript{20} through the TCA cycle in prostate epithelial cells derived from transgenic mice of different genotypes, by using 13C stable-isotope-labeled glucose, glutamine and palmitate. This analysis showed higher glucose and glutamine incorporation into citrate, fumarate and malate in Pten\textsuperscript{pc−/−} cells than in normal prostate epithelial cells. The glucose incorporation into citrate in Pten\textsuperscript{pc−/−}; Pdha1\textsuperscript{pc−/−} tumor cells, was lower (~70%) than that in Pten\textsuperscript{pc−/−} cells. Importantly, PDC inactivation led to compensatory oxidative glutaminolysis and to the production of acetyl CoA from fatty acid beta-oxidation for the reactivation of the TCA (as reflected in a 30% increase in citrate labeling from palmitate). However, this compensation was not able to overcome the decreased citrate synthesis (Supplementary Fig. 4a and Supplementary Table 4). Unexpectedly, glutaminolysis restored the oxidative reactions after α-ketoglutarate to fumarate and malate until aspartate synthesis in TCA cycle. However, the glutamine carbon pool was blocked from the reactions for citrate production, probably because of the lower expression of Idh1 in Pten\textsuperscript{pc−/−}; Pdha1\textsuperscript{pc−/−} than in Pten\textsuperscript{pc−/−} tumor cells (Supplementary Fig. 5dg and Supplementary Tables 5–7). Finally, the pyruvate carboxylase activity did not change in response to PDHA1 loss, because the M + 3 (isotopomer species with three labeled carbons) aspartate was not higher in Pten\textsuperscript{pc−/−}; Pdha1\textsuperscript{pc−/−} than in Pten\textsuperscript{pc−/−} tumor cells (Supplementary Fig. 4g and Supplementary Table 5). Together, these data demonstrated that PDC inactivation decreases the production of TCA intermediates and energetic yield in prostate tumors.

Pdha1 knockout induces tumor suppression by abrogating lipogenesis. Pdha1 has long been defined as a mitochondrial-metabolism regulator. However, recent evidence has demonstrated that PDC controls the nuclear pool of acetyl CoA, thereby promoting histone acetylation and regulating gene expression\textsuperscript{24,25}. In line with this evidence, we detected strong nuclear localization of PDHA1 in Pten\textsuperscript{pc−/−} tumors and PDC activity in both the cytosol and nucleus in these tumor cells (Fig. 1d, Supplementary Figs. 1c and 5ab and uncropped blots in Supplementary Fig. 14). We also detected Pdha1, Dlat and Dld in the nuclear fractions of Pten\textsuperscript{pc−/−} tumor cells (Supplementary Fig. 5c and uncropped blots in Supplementary Fig. 14). Transcriptomics followed by gene set enrichment analysis (GSEA) in Pten\textsuperscript{pc−/−} and Pten\textsuperscript{pc−/−}; Pdha1\textsuperscript{pc−/−} tumors demonstrated that fatty acid synthesis, cholesterol biogenesis and expression of genes controlled by the SREBF25–28 were notably low (ACLY)\textsuperscript{29,30} and squalene epoxidase (SQLE)\textsuperscript{31,32}, was notably low in Pten\textsuperscript{pc−/−}; Pdha1\textsuperscript{pc−/−} tumors (Fig. 2a). Inactivation of Pdha1 in Pten\textsuperscript{pc−/−} tumors and shPDHA1 human prostate cancer cells decreased the levels of acetylated histone H3 Lys9 (H3K9ac), thereby potentially affecting gene expression (Fig. 2bc and uncropped blots in Supplementary Fig. 12). Notably, the expression of two rate-limiting enzymes engaging fatty acid synthesis and cholesterol biogenesis, ATP citrate lyase (ACYL)\textsuperscript{33,34} and squalene epoxidase (SQLE)\textsuperscript{33,35}, was notably low in both Pten\textsuperscript{pc−/−}; Pdha1\textsuperscript{pc−/−} tumors and shPDHA1 human prostate cancer cell lines at both the protein and gene levels (Fig. 2b–f).
Downregulation of ACLY in these cells was not associated with the upregulation of acyl CoA synthetase short-chain family member 2 (ACSS2) in Pten−/−; Pdha1−/− and Pten−/− prostate tumors. DAPI nuclear stain, blue (n=3 mice; scale bars, 20 μm; 1 tumor per mouse; 5 fields acquired). a, Representative confocal images (a) and quantification of average lipid droplets per cell (Lipidtox, red) (b) in the indicated genotypes in Pten−/−; Pdha1−/− and Pten−/− prostate tumors. DAPI nuclear stain, blue (n=3 mice; scale bars, 20 μm; 1 tumor per mouse; 5 fields acquired). b, Representative confocal images (c) and quantification of average lipid droplets per cell (Lipidtox, red) (d) in 22Rv1 and PC3 shPDHA1 and shRNA xenograft tumors. DAPI, blue (n=3 mice; scale bars, 20 μm; 1 tumor per mouse; 5 fields acquired). c, Relative cell-number quantification by crystal violet staining (e) and quantification of average number of lipid droplets per cell (f) in 22Rv1 and PC3 cells infected with a shPDHA1 and scrambled-shRNA control and treated with exogenous fatty acids in fatty acid–free medium; oleate (25 μM), palmitate (25 μM) or a combination of both were used (n=3 independent cell cultures). Error bars, s.e.m. *P<0.05; **P<0.01; ***P<0.001; NS, not significant. P values were determined by ANOVA with Bonferroni correction, unless otherwise noted. Box plots show the median of the distances; the bottom and top of each box represent the first and third quartiles. The lengths of the whiskers extend to 1.5 times the interquartile range unless otherwise noted.

Fig. 3 | Pdha1 knockout induces tumor suppression by abrogating lipogenesis. a, Representative confocal images (a) and quantification of average lipid droplets per cell (Lipidtox, red) (b) in the indicated genotypes in Pten−/−; Pdha1−/− and Pten−/− prostate tumors. DAPI nuclear stain, blue (n=3 mice; scale bars, 20 μm; 1 tumor per mouse; 5 fields acquired). c, Representative confocal images (c) and quantification of average lipid droplets per cell (Lipidtox, red) (d) in 22Rv1 and PC3 shPDHA1 and shRNA xenograft tumors. DAPI, blue (n=3 mice; scale bars, 20 μm; 1 tumor per mouse; 5 fields acquired). d, Relative cell-number quantification by crystal violet staining (e) and quantification of average number of lipid droplets per cell (f) in 22Rv1 and PC3 cells infected with a shPDHA1 and scrambled-shRNA control and treated with exogenous fatty acids in fatty acid–free medium; oleate (25 μM), palmitate (25 μM) or a combination of both were used (n=3 independent cell cultures). Error bars, s.e.m. *P<0.05; **P<0.01; ***P<0.001; NS, not significant. P values were determined by ANOVA with Bonferroni correction, unless otherwise noted. Box plots show the median of the distances; the bottom and top of each box represent the first and third quartiles. The lengths of the whiskers extend to 1.5 times the interquartile range unless otherwise noted.

Downregulation of ACLY in these cells was not associated with the upregulation of acyl CoA synthetase short-chain family member 2 (ACSS2) in Pten−/−; Pdha1−/− and Pten−/− prostate tumors. DAPI nuclear stain, blue (n=3 mice; scale bars, 20 μm; 1 tumor per mouse; 5 fields acquired). Genes that divert TCA-cycle and glutamine intermediates into lipid-metabolic pathways were also downregulated by PDHA1 inactivation in both mouse and human prostate tumor cells (Supplementary Fig. 5d–f). Importantly, acetate supplementation (Supplementary Fig. 5h,i and Fig. 2g,h). Acetate supplementation restored cell growth in both Pten−/−; Pdha1−/− MEFs and shPDHA1 22Rv1 and PC3 cells (Fig. 2i–k). Notably, we did not detect any changes in H3K9ac at the promoters of the cell-cycle regulators E2F transcription factor 1 (E2F1) and cyclin D1 (CCND1), thus demonstrating the specificity of the epigenetic regulation of PDC in cancer cells (Supplementary Fig. 5j,k). Interestingly, ACLY overexpression in prostate cell lines infected with shPDHA1 rescued the growth arrest induced by loss of PDHA1 only in cells supplemented with citrate (Supplementary Fig. 5l–n and uncropped blots in Supplementary Fig. 14), in agreement with previously reported data.
Fig. 4 | Nuclear PDC regulates the expression of lipid-biosynthesis genes independently of mitochondrial PDC. **a**, Western blot analysis of the indicated proteins in nuclear and cytoplasmic fractions of shPDHA1 22Rv1 cells infected with NES-PDHA1 and NLS-PDHA1 alone or in combination. Uncropped images are in Supplementary Fig. 13 (n = 3 independent cell cultures). EV, empty vector. **b**, Western blot analysis of the indicated proteins in shPDHA1 22Rv1 cells infected with NES-PDHA1 and NLS-PDHA1 alone or in combination (full panel in Supplementary Fig. 5b). Uncropped images are in Supplementary Fig. 13 (n = 3 independent cell cultures). **c–e**, qRT-PCR analysis of mRNA expression for ACLY (c) and SQLE (d) and determination of citrate levels (e) in shRNA-control and shPDHA1 22Rv1 and PC3 cells infected with NES-PDHA1 and NLS-PDHA1 alone or in combination (n = 3 independent cell cultures). **f**, Representative confocal images and quantification of lipid droplets (average lipid droplets per cell) in shPDHA1 22Rv1 cells infected with NES-PDHA1 and NLS-PDHA1 alone or in combination (n = 3 independent cell cultures; scale bars, 10 µm; 5 fields acquired per group). **g**, Top, representative images of crystal violet staining of shPDHA1 22Rv1 cells infected with NES-PDHA1 and NLS-PDHA1 alone or in combination (n = 3 independent cell cultures). Bottom, relative cell-number quantification by crystal violet staining in shRNA-control and shPDHA1 22Rv1 and PC3 cells infected with NES-PDHA1 and NLS-PDHA1 alone or in combination (n = 3 independent cell cultures). **h**, Top, representative micrographs in histopathological analysis of Ki-67 in these xenografts. Bottom, evaluation of tumor formation in xenotransplantation experiments of shPDHA1 22Rv1 cells infected with NES-PDHA1 and NLS-PDHA1 alone or in combination (n = 6 animals; 12 injections; scale bars, 50 µm; full panel in Supplementary Fig. 7d). Error bars, s.e.m.; *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. P values were determined by ANOVA with Bonferroni correction, unless otherwise noted.
Fig. 5 | Nuclear PDC regulates fatty acid synthesis in the presence of mitochondrial citrate. a, b, Relative cell-number quantification by crystal violet (a) (full panel in Supplementary Fig. 8a) and quantification by confocal microscopy of average lipid droplets per cell (b) in shPDHA1 22Rv1 and PC3 cells infected with NES-PDHA1 and NLS-PDHA1 alone or in combination and treated with citrate (100 μM) or vehicle for 6 d (n = 3 independent cell cultures). The box plots show the median of the distances; the bottom and top of each box represent the first and third quartiles. The lengths of the whiskers extend to 1.5 times the interquartile range unless otherwise noted. c, Top, representative confocal images and quantification of average lipid droplets per cell in xenograft tumors from shRNA-control and shPDHA1 tumors infected with PDK1 or empty vector. Uncropped images are in Supplementary Fig. 13 (n = 6 independent tumor samples). Error bars, s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. P values were determined by ANOVA with Bonferroni correction, unless otherwise noted.

We next performed a direct measurement of lipid species in mouse prostate tumors and human prostate cancer cell lines depleted of PDHA1. Notably, most lipid species, including cholesterol esters, were lower in Pten<sup>p<sup>-/-</sup></sup>; Pdha1<sup>p<sup>-/-</sup></sup> tumors than in Pten<sup>p<sup>-/-</sup></sup> tumors and in shPDHA1 22Rv1 and PC3 cells than in control cells (Supplementary Fig. 6, Supplementary Tables 8–11 and Supplementary Note). <sup>14</sup>C tracking experiments demonstrated that glucose and glutamate incorporation into lipids and cholesterol (Supplementary Fig. 7a–e and Supplementary Note) were strongly affected in shPDHA1 22Rv1 and PC3 cells compared with controls, whereas glucose and glutamine incorporation into proteins remained unchanged (Supplementary Fig. 7f,g and Supplementary Note). PDHA1 knockdown did not affect glucose uptake but induced a slight increase in glutamine uptake (Supplementary Fig. 7h,i and Supplementary Note). In agreement with these findings, lipid droplets, a known indicator of lipid production<sup>5,37</sup>, were

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markedly more scarce in both mouse and human xenograft tumors after PDHA1 inactivation (Fig. 3a–d and Supplementary Fig. 7j–o). To assess whether lipid metabolism was affected by PDHA1 inactivation, we cultured shPDHA1 22Rv1 and PC3 cells in the presence or absence of fatty acids, and we found that fatty acids fully rescued the growth arrest induced by the PDHA1 knockdown. This observation was associated with the restoration of lipid droplets in the same cells (Fig. 3e,f and Supplementary Note). Rescue of proliferation by fatty acid supplementation occurred independently through H3K9 acetylation and reactivation of the expression of ACLY and SQLE (Supplementary Fig. 7p and uncropped blots in Supplementary Fig. 15). These data suggested that fatty acids were directly incorporated into lipids. Collectively, our results demonstrated that PDC activity is required for the proliferation of mouse and human prostate tumor cells and that inactivation of PDC drives tumor growth inhibition by affecting H3K9 acetylation and the expression of genes controlling lipid metabolism.

Nuclear PDC regulates expression of lipid-biosynthesis genes independently of mitochondrial PDC. Cellular compartmentalization allows cells to carry out different metabolic reactions simultaneously, and pathological protein compartmentalization is associated with cancer13. We therefore investigated the roles of nuclear and cytosolic PDC in prostate cancer, by expressing nuclear export signal (NES)- and/or nuclear localization signal (NLS)-fused PDHA1 vectors in prostate cancer cell lines previously infected with shPDHA1 or left uninfected (Fig. 4a and uncropped blots in Supplementary Figs. 8a and 13). NES-PDHA1 and NLS-PDHA1 constructs were engineered as shPDHA1-resistant vectors. Expression of these vectors in 22Rv1 cells did not affect SREBF1 maturation or nuclear translocation (Supplementary Fig. 8a). Restoration of NLS-PDHA1 in shPDHA1 cells restored H3K9ac and the expression of fatty-acid-synthesis genes (Fig. 4b–d, full panel in Supplementary Figs. 8b and uncropped blots in Supplementary Figs. 13 and 15) but did not rescue citrate levels, lipid content or cell growth both in vitro and in vivo (Fig. 4e–i and Supplementary Fig. 8c–g). In contrast, overexpression of both NES-PDHA1 and NLS-PDHA1 restored lipid-synthesis-gene expression, citrate levels, lipid content and cell growth (Fig. 4b–d and Supplementary Fig. 8b–g). Interestingly, the selective ACLY inhibitor SB-204990 partially decreased H3K9ac levels in prostate cancer cells with high PDC activity but did not further increase the growth arrest in prostate cancer cells infected with a shPDHA1 (Supplementary Fig. 8h–j and uncropped blots in Supplementary Fig. 15). These data demonstrated that PDC directly contributes to H3K9 acetylation, but not by ACLY16. These experiments show that nuclear PDC regulates the expression of lipid-synthesis genes in an autonomous manner, and both nuclear and cytosolic PDHA1 are needed for the proliferation of prostate cancer cells.

Nuclear PDC regulates fatty acid synthesis in the presence of mitochondrial citrate. In agreement with these findings, we showed that overexpression of NLS-PDHA1 rescued growth arrest and lipid synthesis in cells cultured in the presence of citrate (Fig. 5a,b and full panel in Supplementary Fig. 9a). To validate these findings by using an additional system, we overexpressed PDHK1 in prostate cancer cells. Previous evidence has demonstrated that PDHK1 overexpression suppresses mitochondrial metabolism, thus decreasing the intracellular levels of citrate without halting the nuclear function of PDC19,39–41. In line with this evidence, overexpression of PDHK1 did not promote phosphorylation of nuclear PDHA1 (Supplementary Fig. 9b and uncropped blots in Supplementary Fig. 15). Overexpression of PDHK1 in 22Rv1 cells decreased citrate levels and slightly suppressed cancer cell proliferation both in vitro and in vivo (Fig. 5c–e and Supplementary Fig. 8c–e); however, it did not decrease histone acetylation and expression of fatty-acid-synthesis genes (Fig. 5f; uncropped blots in Supplementary Fig. 13 and Supplementary Fig. 9b). In contrast, concomitant inactivation of PDHA1 and overexpression of PDHK1 decreased citrate levels, histone acetylation and lipid content, thereby suppressing tumorigenesis to a greater extent than that in cells infected with PDHK1 alone (Fig. 5c–f and Supplementary Fig. 9c–e). Together, these results demonstrated that both mitochondrial and nuclear PDC is required for prostate tumor growth.

Pharmacological inhibition of PDHA1 arrests mouse and human prostate tumors. Given that prostate cancer relies on PDC for proliferation, we assessed whether pharmacological inhibition of PDHA1 could also block tumorigenesis in Pten<null> tumors and human xenograft models of prostate cancer. Reasoning that a compound affecting both the mitochondrial and nuclear function of PDC might be effective in blocking prostate cancer, we took advantage of 3-fluoropyruvate (3-FP), a competitive inhibitor of PDHA1 (refs 42–44). Computer simulations indicated that 3-FP and pyruvate bind PDHA1 with similar affinity (Supplementary Fig. 10a,b and Supplementary Note). To assess the specificity of 3-FP for PDC, cell lysates treated with 3-FP were incubated in the presence or absence of pyruvate. Treatment with 3-FP decreased the PDC activity, and this effect was reversed in a dose-dependent manner by addition of pyruvate to the reaction buffer (Supplementary Fig. 10c). Intraperitoneal injection of 3-FP in Pten<null> mice inhibited PDC activity in vivo without causing systemic toxicity and induced a strong decrease in tumor cell proliferation, as demonstrated by the smaller tumor size and the lower tumor invasiveness and Ki-67 staining (Supplementary Fig. 10d–j). Importantly, 3-FP inhibited PDC in both the nucleus and the cytosol in prostate tumor cells and decreased H3K9ac, ACLY and SQLE levels in vivo at both the protein and mRNA levels (Supplementary Fig. 10k–m and uncropped blots in Supplementary Fig. 15). Metabolomic analysis via liquid chromatography–tandem mass spectrometry (LC–MS/MS)45 confirmed that 3-FP strongly affected pyruvate metabolism and the TCA cycle (Supplementary Fig. 11a). Finally, lipid-droplet staining showed decreases in the number, size and staining intensity of lipid bodies in Pten<null> tumors treated with 3-FP (Supplementary Fig. 11b–e). 3-FP treatment blocked the proliferation of different human prostate cancer cells, and this effect was associated with decreased PDC activity (Supplementary Fig. 11f,g). Finally, 3-FP treatment decreased the proliferation of different human prostate cancer xenografts (LNCaP, 22Rv1 and PC3), thus promoting long-lasting antitumor responses in vivo (Supplementary Fig. 11h–j). Together, these data suggest that pharmacological inactivation of PDC hinders prostate cancer progression.

Discussion

Whether PDC promotes or suppresses tumor development is still under debate9,12,23,39,40,47–49. A previous report has shown that decreased PDC activity through overexpression of PDHK1 in melanoma cells promotes tumor cell proliferation by enhancing glycolysis and reductive carboxylation41. In this and other models, activation of PDC by dichloroacetate leads to tumor suppression45. Our data reveal a new angle by demonstrating that PDC subunits, including PDHA1 and PDPI, are amplified and overexpressed at both the gene and protein levels and that PDC is active in prostate cancer. In line with this evidence, we demonstrated that inactivation of PDHA1 hinders prostate cancer progression in vivo in both mouse and human prostate cancer models. These findings are in agreement with those from recent studies demonstrating that tumor cells, in vivo, increase glucose oxidation via PDC. PDHA1 inactivation in non–small cell lung cancer (NSCLC) xenografts decreases tumor formation ability, and upregulation of the TCA cycle via PDC has also been observed in poorly perfused tumor areas in patients with NSCLC44,51. These data, together with our data in
prostate cancer, challenge the notion that tumors switch from glucose-derived oxidative metabolism to aerobic glycolysis to support their growth. However, the mechanisms by which tumor cells in vivo become addicted to PDC-mediated mitochondrial metabolism still remain unknown. As suggested by previous studies, this metabolic dependency may be explained by differences in tumor genetic backgrounds, tissue requirements or the composition of the tumor microenvironment. Intriguingly, normal prostate epithelial cells exhibit a ‘truncated’ TCA cycle, owing to inhibition of aconitase, which is needed for the production and secretion of a high quantity of citrate into the seminal fluid. Therefore, it is not surprising that prostate cancer relies on mitochondrial metabolism more than do other tumor types.

Our results indicate that to support prostate cancer growth, PDC must be functional in both the mitochondria and the nuclei in cancer cells. Mitochondrial PDC function is not sufficient to support lipid biosynthesis in tumor cells, because nuclear PDC regulates the transcription of the enzymes that convert cytosolic citrate into acetyl CoA and fatty acids (Supplementary Fig. 11k,l). This compartmentalization allows for the mitochondrial production of citrate in the cytosol and the expression of enzymes required for de novo lipid biosynthesis. Prior studies have demonstrated that prostate cancer also benefits from enhanced glycolytic metabolism. However, these aggressive glycolytic tumor cells retain an active glucose-derived lipid biosynthesis, in line with the results presented herein. Our findings also have important implications for potential cancer therapy and may pave the way for the targeting of the nuclear function of PDC to eradicate prostate cancer. Future eradication might be achieved as described in this paper by using pyruvate analogs or alternatively by developing small-molecule inhibitors of PDHA1.

Methods
Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41588-017-0026-3.

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References
1. Zong, W. X., Rabinowitz, J. D. & White, E. Mitochondria and cancer. Mol. Cell 61, 667–676 (2016).
2. LeBlanc, V. S. et al. PGC-1α mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. Nat. Cell. Biol. 16, 992–1003 (2014).
3. Marin-Valencia, I. et al. Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain in vivo. Cell Metab. 15, 827–837 (2012).
4. Vazquez, F. et al. PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. Cancer Cell 23, 287–301 (2013).
5. Viale, A. et al. Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. Nature 514, 628–632 (2014).
6. Ahn, C. S. & Metallo, C. M. Mitochondria as biosynthetic factories for cancer proliferation. Cancer Metab. 3, 1 (2015).
7. Weinberg, S. E. & Chandel, N. S. Targeting mitochondria metabolism for cancer therapy. Nat. Chem. Biol. 11, 9–15 (2015).
8. Vyas, S., Zaganjon, E. & Haigis, M. C. Mitochondria and cancer. Cell 166, 555–566 (2016).
9. Hensley, C. T. et al. Metabolic heterogeneity in human lung tumors. Cell 164, 681–694 (2016).
10. Davidson, S. M. et al. Environment impacts the metabolic dependencies of Ras-driven non-small cell lung cancer. Cell Metab. 23, 517–528 (2016).
11. Wieland, O. H. The mammalian pyruvate dehydrogenase complex: structure and function. Cell Metab. 15, 827–837 (2012).
12. Kolobova, E., Tuganova, A., Boulatnikov, I. & Popov, K. M. Regulation of pyruvate dehydrogenase activity through phosphorylation at multiple sites. Biochem. J. 358, 69–77 (2001).
13. Roche, T. E. et al. Distinct regulatory properties of pyruvate dehydrogenase kinase and phosphatase isoforms. Prog. Nucleic Acid Res. Mol. Biol. 70, 33–75 (2001).
14. Tromtan, L. C. et al. Pten dose dictates cancer progression in the prostate. PLoS Biol. 1, E59 (2003).
15. Chen, Z. et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 436, 725–730 (2005).
16. Milowska, A. et al. Subtle variations in Pten dose determine cancer susceptibility. Nat. Genet. 42, 454–458 (2010).
17. Wu, X. et al. Generation of a prostate epithelial cell-specific Cre transgenic mouse model for tissue-specific gene ablation. Mech. Dev. 101, 61–69 (2001).
18. Johnson, M. T. et al. Inactivation of the murine pyruvate dehydrogenase (Pdha1) gene and its effect on early embryonic development. Mol. Genet. Metab. 74, 293–302 (2001).
19. Kaplon, J. et al. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature 498, 109–112 (2013).
20. Vacanti, N. M. et al. Regulation of substrate utilization by the mitochondrial pyruvate carrier. Mol. Cell 56, 425–435 (2014).
21. Yang, C. et al. Glutamine oxidation maintains the TCA cycle and cell survival during impaired mitochondrial pyruvate transport. Mol. Cell 56, 414–424 (2014).
22. Rajagopalan, K. N. et al. Metabolic plasticity maintains proliferation in pyruvate dehydrogenase deficient cells. Cancer Metab. 3, 7 (2015).
23. Sutendra, G. et al. A nuclear pyruvate dehydrogenase complex is important for the generation of acetyl-CoA and histone acetylation. Cell 158, 84–97 (2014).
24. Nagaraj, R. et al. Nuclear localization of mitochondrial TCA cycle enzymes as a critical step in mammalian zygotic genome activation. Cell 168, 210–223 (2017). e211 (2017).
25. Briggs, M. R., Yokoyama, C., Wang, X., Brown, M. S. & Goldstein, J. L. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. J. Biol. Chem. 268, 14490–14496 (1993).
26. Wang, X. et al. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. II. Purification and characterization. J. Biol. Chem. 268, 14497–14504 (1993).
27. Porstmann, T. et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab. 8, 224–236 (2008).
28. Han, J. et al. The CREB coactivator CRTC2 controls hepatic lipid metabolism by regulating SREBP1. Nature 524, 243–246 (2015).
29. Hatzivassiliou, G. et al. ATC citrate lyase inhibition can suppress tumor cell growth. Cancer Cell 8, 311–321 (2005).
30. Welten, K. E. et al. ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324, 1076–1080 (2009).
31. Helms, M. W. et al. Squalene epoxidase, located on chromosome 8q24.1, is upregulated in 8q breast cancer and indicates poor clinical outcome in stage I and II disease. Br. J. Cancer 99, 774–780 (2008).
32. Santiniano, J., Luu, W., Kristiana, I. & Brown, A. J. Squalene mono-oxygenase, a key enzyme in cholesterol synthesis, is stabilized by unsaturated fatty acids. Biochem. J. 415, 435–442 (2014).
33. Zhao, S. et al. ATP-citrate lyase controls a glucose-to-acetate metabolic switch. Cell Rep. 17, 1037–1052 (2016).
34. Bulusu, V. et al. Acetate recapturing by nuclear acetyl-CoA synthetase 2 prevents loss of histone acetylation during oxygen and serum limitation. Cell Rep. 18, 647–657 (2017).
35. Metallo, C. M. et al. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature 481, 385–388 (2011).
36. Carne, A. A. et al. The reductive glutamine metabolism pathway links mitochondrial metabolism to liver cancer. Cell Metab. 18, 153–161 (2013).
37. Aguzzoli, A. & Almeyrre, M. Phase separation: linking cellular compartmentalization to disease. Trends Cell Biol. 26, 547–558 (2016).
38. Kim, J. W., Tchernyshyov, I., Semenza, G. L. & Dang, C. V. HIF-1-mediated expression of pyruvate kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 3, 177–185 (2006).
39. Papandreou, I., Cairns, R. A., Fontana, L., Lim, A. L. & Denko, N. C. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab. 3, 187–197 (2006).
40. Degenhardt, P. J. et al. PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. Cell Metab. 22, 577–589 (2015).
41. Mager, J. & Blank, I. Synthesis of fluoropyruvic acid and some of its biological properties. Nature 173, 126–127 (1954).
ARTICLES

43. Avi-Dor, Y. & Mager, J. The effect of fluoropyruvate on the respiration of animal-tissue preparations. Biochem. J. 63, 613–618 (1956).

44. Chari-Bitron, A. & Avi-Dor, Y. Effect of fluoropyruvate on the swelling, phosphorylative activity and respiration of guinea-pig liver mitochondria. Biochem. J. 71, 572–578 (1959).

45. Du, J. et al. Inhibition of mitochondrial pyruvate transport by zarnipant causes massive accumulation of aspartate at the expense of glutamate in the retina. J. Biol. Chem. 288, 36129–36140 (2013).

46. Yuan, M., Breitkopf, S. B., Yang, X. & Asara, J. M. A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. Nat. Protoc. 7, 872–881 (2012).

47. Hitosugi, T. et al. Tyrosine phosphorylation of mitochondrial pyruvate dehydrogenase kinase 1 is important for cancer metabolism. Mol. Cell 44, 864–877 (2011).

48. Kerr, E. M., Gaude, E., Turrell, F. K., Frezza, C. & Martins, C. P. Mutant Kras channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11, 37–51 (2007).

49. Bonnet, S. et al. A mitochondria K+-channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11, 37–51 (2007).

50. Vander Heiden, M. G., Cantley, L. C. & Thompson, C. B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324, 1029–1033 (2009).

51. Costello, L. C. & Franklin, R. B. Aconitase activity, citrate oxidation, and zinc inhibition in rat ventral prostate. Enzyme 26, 281–287 (1981).

52. Costello, L. C., Liu, Y., Franklin, R. B. & Kennedy, M. C. Zinc inhibition of mitochondrial aconitase and its importance in citrate metabolism of prostate epithelial cells. J. Biol. Chem. 272, 28875–28881 (1997).

53. Costello, L. C., Liu, Y., Zou, J. & Franklin, R. B. The pyruvate dehydrogenase E1 alpha gene is testosterone and prolactin regulated in prostate epithelial cells. Endocr. Res. 26, 23–39 (2000).

54. Torrano, V. et al. The metabolic co-regulator PGC1alpha suppresses prostate cancer metastasis. Nat. Cell Biol. 18, 645–656 (2016).

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Author contributions

J.C. and A. Alimonti conceived the project, designed the experiments, interpreted the data and wrote the paper: J.C., J.G., D.D.M., D.B., A. Revandkar and A. Alajati performed experiments and analyzed the data. J.C. performed crosses and generated the transgenic mouse model. G.C., E.P., C.V.C. and J.C. performed experiments on the xenograft mouse model. A. Alajati, S.P., M.L., M.S. and J.d.B. performed immunohistochemical experiments and analysis. R.D. and J.C. established and carried out fluorescence microscopy, J.M.A. and J.C. conducted metabolic and lipidomic analysis on mouse prostate tumors. S.C., M.A., M.M. and J.C. performed cholesterol measurements in prostate cancer cell lines. A.M. and P.C. performed 14C tracing experiments and interpreted the data. I.M. and A. Rasola carried out mitochondrial OCR measurements. J.S. and A. Cavali conducted computational structural analysis. A. Rinaldi, N.D. and F.B. performed gene expression profiling on mouse tumors and GSEA analysis of metabolic pathways on mouse tumors. R.G.-E. provided bioinformatic analysis on PDHA1 and PDP1 amplification and overexpression in human prostate cancer datasets. A. Carracedo provided support on metabolic analysis, interpretation of results and discussion.

Competing interests

The authors declare no competing financial interests.

Additional information

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Methods

Animals. All mice were maintained under specific-pathogen-free conditions in the animal facilities of the IRB Institute, and experiments were performed according to state guidelines and were approved by the Swiss ethics committee under authorization number 04-2017. The Pten<sup>−/−</sup> and Pdha1<sup>−/−</sup> conditional knockout alleles have been previously described<sup>15,18</sup>. Female Pten<sup>−/−</sup> and Pdha1<sup>−/−</sup> mice were crossed with male Pseudobas-Cre (Pc-Cre) transgenic mice<sup>16</sup> for the prostate-specific deletion of Pten and Pdha1. For genotyping, tail DNA was subjected to polymerase chain reaction analysis with the primers listed in Supplementary Table 1. The mice of the indicated genotypes were randomly chosen and allocated into experimental groups. For the transgenic mice, the experiment was carried out in a single-blinded manner.

Cell culture and reagents. Human prostate carcinoma cell lines 22Rv1, LNCaP, and PC3 (purchased from the ATCC); and the human prostate epithelial PNT2C2 cell line<sup>16</sup> (a gift from J.M. Maitland, University of York) were kept in the Institute of Oncology in Belinovina, Belgrade. The cells were cultured in RPMI medium 1640 (21875034, Thermo Fisher Scientific) with 10% FBS (10500-064, Thermo Fisher Scientific), penicillin (10,000 IU/ml) and streptomycin (10,000 µg/ml; P4333-20ML, Sigma) under 37 °C and 5% CO<sub>2</sub>. Cells were transduced with the Tripz doxycline-inducible lentiviral construct against the human PDHA1 gene (V2THS_57677, Dharmacon) and TriPz Inducible Lentiviral Empty Vector shRNA Control (RHS4750, Dharmaco). Flat-bottom ultra-low-attachment multidwell plates (3473, Corning) were used for sphere formation assays. Cells were transduced with pLKO lentiviral constructs against the human PDAH1 gene (SHCLNg-NM_002824, Sigma, clone shPDAH1-1, TRCN00028852, and TRCN00028853) or empty vector. Tripz Inducible Lentiviral Empty Vector shRNA Control (SHC002, Sigma). PDAH1 was PCR-amplified from complementary DNA from a normal human prostate sample. For the overexpression of cytosolic- and nuclear-localized PDHA1, an NES or NLS was fused with PDHA1, thus yielding NES-PDHA1 and NLS-PDHA1, respectively. NES-PDHA1 and NLS-PDHA1 constructs were engineered as shPDHA1 (SHCLNg-NM_002824, Sigma, clone shPDHA1-1, TRCN00028852)-resistant vectors. NES-PDHA1 and NLS-PDHA1 were cloned into the plenti CMV Neo (Addgene) vector, and empty vector was used as a control. Cells were infected with shRNA control or shPDHA1, and PDK1 was overexpressed through pLZRS-PDK1-IRES-puro or FG12-eGFP control<sup>19</sup> (gift from D. Peeper, Netherlands Cancer Institute). Lenti- and retroviral infections were performed with HEK293T cells and Phoenix cells, respectively, as producers of viral supernatants. Primary MEFs were cultured in RPMI medium 1640 (21875034, Thermo Fisher Scientific) with 10% FBS (10500-064, Thermo Fisher Scientific), penicillin (10,000 IU/ml) and streptomycin (10,000 µg/ml; P4333-20ML, Sigma) under 37 °C and 5% CO<sub>2</sub>. Lentiviral and retroviral infections were performed with HEK293T cells and Phoenix cells, respectively, as producers of viral supernatants.

Gene expression analysis. Gene expression profiling (GEP) was done with MouseRef-8 v2.0 Expression BeadChips (Illumina), according to the manufacturer's protocol. Arrays were read on an Illumina HiScanSQ system. Data were first extracted with Illumina GenomeStudio software and then imported into Genomics Suite 6.4 (Partek Incorporated) and quantile normalized. Transcripts with differences in expression were identified through ANOVA. Enrichment analysis was performed through GSEA<sup>9</sup>. Raw data have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) database under accession number GSE4245. GSEA was performed on an entire gene list ranked according to fold changes observed between Pten<sup>−/−</sup> and Pdha1<sup>−/−</sup> animals. The GSE collection assessed included all GSs smaller than 10 and larger than 500 (8,335 out of 22,423 GSs retained), compiled according to ref. 5. The 24.03.15. GSs yielding significance (FDR <0.05; nominal P value <0.005; TAGS ≥50%) were retained and assessed for their role in metabolic processes after the data were significance described<sup>5,18</sup>. For the three relevant clusters identified, their GS FDR q values, together with unaffected metabolic processes, were log-transformed and reversed, as displayed in Fig. 2a.

Chromatin immunoprecipitation. ChIP assays were performed with approximately ×10<sup>6</sup> cells per experiment. Cells were subjected to hypotonic lysis and treated with micrococcal nuclease to recover mono- to trinucleosomes. Nuclei were lysed by brief sonication and dialyzed against N-ChIP buffer (10 mM Tris, pH 7.6, 1 mM EDTA, 0.1% SDS, 0.1% sodium deoxycholate and 1% Triton X-100) for 2 h at 4 °C. Soluble material was incubated overnight at 4 °C after the addition of 5–1 µg of antibody bound to 25 µl Protein A Dynal magnetic beads (10006D, Invitrogen), and 5% was kept as input DNA. The magnetic beads were washed, chromatin was eluted, and ChIP DNA was dissolved in 10 mM Tris, pH 8, for qPCR reactions (described below). Three separate ChIP experiments were performed on replicate biological samples. The data shown are average qRT–PCR values (+ S.E.M.). Primers are listed below. qPCR was performed with an Applied BiosystemsStepOnePlus system and Power SYBR Green PCR master mix. ChIP samples were diluted 1:100 in H<sub>2</sub>O, and 5 µl was used per reaction. ChIP–qPCR signals were calculated as percentage input. Primers used in ChIP are listed in Supp. Table 3.

Subcellular fractionation. Nuclear and cytoplasmic fractionation was performed through a centrifugation technique as described previously<sup>34</sup>. Nuclear and cytoplasmic extracts were made with an NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (78833, Pierce Biotechnology). Total cell lysate was prepared in radioimmunoprecipitation assay buffer (RIPA; 25 mMol/l Tris, pH 7.4, 150 mMol/l KCl, 5 mMol/l EDTA, 1% NP-40, 0.5% sodium deoxycholate and 0.1% protease-inhibitor cocktail (88688, Thermo Scientific)). All proteins were separated by 10–15% SDS–PAGE and transferred onto polyvinylidene difluoride membranes. For each fraction, the nuclear protein histone 3 (4499, Cell Signaling Technology) and the cytoplasmic protein α-tubulin (2144, Cell Signaling Technology) were used to show clear isolation between nucleus and cytoplasm during fractionation.

Targeted mass spectrometry analysis. For mouse prostate tissues, 500 µl of 80% LC–MS-grade methanol was added to each sample of approximately 15 mg and
incubated at −80 °C for 15 min. Tissue samples were centrifuged at 18,470 g for 5 min in a cold room to pellet cell debris and proteins. Supernatants were saved. Pellets were resuspended in 500 μl 80% methanol by vortexing, then centrifuged as before. Supernatants were centrifuged one final time at 18,470 g for 10 min at 4 °C. Metabolite extractions were dried to a pellet in a SpeedVac rotary evaporator with no heat. Samples were resuspended in 20 μl LC–MS-grade water, and 10 μl was injected and analyzed with a 5500 QTRAP hybrid triple-quadrupole mass spectrometer (AB/SCIEX) coupled to a Prominence UFLC HPLC system (Shimadzu) through selected reaction monitoring (SRM). Endogenous water-soluble metabolites (n = 261) were targeted for steady-state analyses of samples. Some metabolites were targeted in both positive- and negative-ion modes through positive/negative polarity switching for a total of 296 SRM transitions. The ESI voltage was +4,900 V in positive-ion mode and −4,500 V in negative-ion mode. The dwell time was 3 ms per SRM transition, and the total cycle time was ~1.56 s.

Approximately 10–12 data points were acquired per detected metabolite. Samples were delivered to the MS through normal-phase chromatography with a 4.6-mm i.d. × 10 cm Amide XBridge HILIC column (Waters Corp.) at 350 μl/min. Gradients were run as follows: 85% buffer B (HPLC-grade acetonitrile) to 35% B from 0 to 3.5 min; 35% B to 2% B from 3.5 to 11.5 min; 2% B held from 11.5 to 16.5 min; 2% B to 85% B from 16.5 to 17.5 min; 85% B held for 7 min to reequilibrate the column. Buffer A comprised 20 mM ammonium hydroxide and 20 mM ammonium acetate, pH 9.5, in 95.5% water/acetonitrile. Peak areas from the total ion current for each metabolite SRM transition were integrated in MultiQuant v2.1 software (AB/SCIEX). Metabolomic data analysis was done in part in Metaboanalyst 2.0 software (URLs).

Isotope labeling and stationary profiling. Fresh transgenic mouse tumor tissues were mechanically dissociated, enzymatically digested and filtered to obtain single-cell suspensions as previously described. Single cells were stained with fluorescein isothiocyanate (FITC)–anti-CD45 (553079, BD Bioscience) isothiocyanate (FITC)–anti-CD34 (560238, BD Biosciences) for stroma cells, FITC–anti-CD45 (553079, BD Bioscience) for endothelial cells and FITC–anti-CD45 (553079, BD Bioscience) for leukocytes, and were incubated 20 min on ice. All antibodies (BD Biosciences) were used at 1:300; cells were then loaded into an MS column with anti-FITC Microbeads (130-048-701, Miltenyi Biotec) for MACS separation, and unstained epithelial cells were collected in the negative fraction. For steady-state metabolomic analysis, prostate epithelial cells derived from transgenic mouse tumors were plated to ∼80% confluence on 10-cm dishes in biological quadruplicate. Cells were plated in RPMI medium (1187093, Thermo Scientific) supplemented with 10% dialsed serum, devoid of glucose or glutamine, and containing one of the two 13C-labeled substrates [U-13C6]glucose and [U-13C5]glutamine (CLM-1396-1 and CLM-1396-2) at 5 mM each, or unlabeled (11 mM glucose and 2 mM glutamine). Fatty acid oxidation studies were conducted with [1-13C16]palmitate (CLM-6059-1, Cambridge Isotope Labs) noncovalently bound to fatty acid–free BSA. [U-13C5]palmitate-BSA was added to the culture medium at 5% of the final volume (50 mM final concentration) with 1 mM carnitine in medium formulated with delipidated FBS (1276001, Thermo Scientific). Additionally, fresh medium containing 13C-labeled substrates was exchanged 2 h before metabolite extraction for steady-state analyses. Because primary epithelial cells isolated from mouse prostate tissue do not attach and are thus unsuitable for long-term culture, we labeled these epithelial cells for 6 h, and we observed no differences compared with the results of labeling for 24 h. After 6 h of incubation with labeled substrates, metabolite extraction of the medium was performed by addition of 1 ml cold (~80 °C) 80% methanol (34966-1 and 14263-1, Sigma), incubation at ~80 °C for 30 min and centrifugation at 10,000 g for 10 min at 20 °C. The resultant supernatant was lyophilized in a SpeedVac and stored at −80 °C until analysis. Dried metabolite pellets were resuspended in 20 μl LC–MS-grade water, and 5 μl was injected onto a Prominence UFLC and separated with a 4.6-mm i.d. × 100 mm Amide XBridge HILIC column at 360 μl/min starting from 85% buffer B (100% ACN) to 0% B over 16 min. Buffer A consisted of 20 mM NH4OH and 20 mM CH3COONH4, pH 9.0, in 95.5:4.5 ACN/ACN. Selected reaction monitoring (SRM) transitions were captured through positive/negative polarity switching by targeted LC–MS/MS with a 5500 QTRAP hybrid triple-quadrupole mass spectrometer. For metabolomics, the quantity of the metabolite fraction analyzed was adjusted to the corresponding protein concentration from a sample processed in parallel. The analysis was performed for each of the three substrates on three independent tumors in biological triplicate.

Lipidomics analysis of transgenic-mouse prostate tumors. Approximately 5 mg of solid tissue was snap frozen in liquid nitrogen (~196 °C) as close to the time of resection as possible. Chloroform/methanol (2:1) was added to a final volume 20 times the volume of the biological sample (100 μl in 2 ml of solvent mixture) in a 12-ml glass vial. The mixture was agitated for 30 min in an orbital shaker at room temperature; then 0.2 volumes of water (e.g., 400 μl for 2 ml) was added, and the mixture was vortexed for 1 min. The mixture was allowed to rest for 10 min and was then centrifuged at low speed (1,000 g) to separate it into three phases. The upper aqueous phase was kept (optionally) to analyze small organic polar molecules. The middle layer contained protein, DNA and large polyelectrolytes. The lower phase containing nonpolar lipids was collected and evaporated under vacuum with a SpeedVac or under a nitrogen stream. 10 μl of sample was injected for LC–MS/MS with a hybrid QExactive Plus Orbitrap mass spectrometer in DDA mode with positive/negative ion-polarity switching (top eight in both modes). With a 100-mm × 2.0-mm C18 column at 260 μl/min with a 1100 quaternary HPLC, lipids were eluted over 20 min from 32% buffer B (90% IPA, 10% can, 10 mM NH4HCO3 and 0.1% FA) to 97% B. Buffer A consisted of 59.9% can, 40% water, 10 mM NH4HCO3, and 0.1% FA. Lipid molecules were identified and quantified with LipidSearch 4.1.9 software.

Statistics. For each independent in vitro experiment, at least three technical replicates were used (exceptions: in western blot analysis, technical replicates were used; in targeted metabolomics, three technical replicates were used). For data-mining analysis, ANOVA tests were used for multicomponent comparisons, and Student’s t tests were used for paired comparisons. In the in vitro experiments, data groups were assessed for normal distribution, and Student’s t test was applied for paired comparisons. Data represent mean ± s.e.m. of pooled experiments unless otherwise stated. n values represent the numbers of experimental samples, and all the experiments were repeated at least three times. For in vivo experiments, because equal variance could not be assumed, a nonparametric Mann–Whitney test was used. The confidence level used for all the statistical analyses was 0.95 (α = 0.05). Two-tailed statistical analysis was applied for experimental designs without predicted results, and one-tailed statistical analysis was applied for validation experiments (*P < 0.05; **P < 0.01; ***P < 0.001).

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. The mouse gene expression datasets are available at NCBI’s Gene Expression Omnibus database under accession number GSE74425. For cellular, molecular and metabolic assays, the lipidomic analysis on prostate cancer cell lines, fatty acid rescue assays and computer simulations of 3-FP and PDC docking, methods are available in the Supplementary Note.

References
55. Matlud, N. J. et al. In vitro models to study cellular differentiation and function in human prostate cancers. Radiat. Res. 155, 133–142 (2001).
56. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. USA 102, 15545–15550 (2005).
57. Merico, D., Isserlin, R., Stueker, O., Emili, A. & Bader, G. D. Enrichment map: a network-based method for gene-set enrichment visualization and interpretation. PLoS One 5, e13984 (2010).
58. Delaleu, N., Nguyen, C. Q., Tekle, K. M., Jonsson, R. & Peck, A. B. Transcriptional landscapes of emerging autoimmunity: transient aberrations in the targeted tissue's extracellular milieu precede immune responses in SJögren's syndrome. Arthritis Res. Ther. 15, R174 (2013).
59. Zhao, J. et al. TIP30 induces apoptosis under oxidative stress through stabilization of p53 messenger RNA in human hepatocellular carcinoma. Cancer Res. 68, 4133–4141 (2008).
60. Lukacs, R. U., Goldstein, A. S., Lawson, D. A., Cheng, D. & Witte, O. N. Isolation, cultivation and characterization of adult murine prostate stem cells. Nat. Protoc. 5, 702–713 (2010).
61. Breitkopf, S. B. et al. A relative quantitative positive/negative ion switching method for untargeted lipidomics via high resolution LC–MS/MS from any biological source. Metabolomics 13, 30 (2017).
## Experimental design

1. **Sample size**
   
   Describe how sample size was determined.

   (Page# 31-32) Methods: Statistics. For each independent in vitro experiment, at least three technical replicates were used (exceptions: in western blot analysis technical replicates are presented, in targeted metabolomics three technical replicates were used. For the xenograft tumour experiment in immune compromised mice, 2×10^6 cells were injected for each tumour, ten sites were injected subcutaneously for each experiment and six tumours of size around the average were counted in the experiments. n values represent the number of experimental samples and all the experiments were repeated at least three times.

2. **Data exclusions**
   
   Describe any data exclusions.

   (Page# 23-25) Methods: Cell culture and reagents. For experiments with xenograft, mice were excluded when the tumour size exceeded the average tumour size at the moment of randomization.

3. **Replication**
   
   Describe whether the experimental findings were reliably reproduced.

   (Page# 31-32) Methods: Statistics. For each independent in vitro experiment, at least three technical replicates were used (exceptions: in western blot analysis technical replicates are presented, in targeted metabolomics three technical replicates were used. n values represent the number of experimental samples and all the experiments were repeated at least three times.

4. **Randomization**
   
   Describe how samples/organisms/participants were allocated into experimental groups.

   (Page# 23-25) Methods: Cell culture and reagents. For experiments with xenograft, mice were excluded when the tumour size exceeded the average tumour size at the moment of randomization.

5. **Blinding**
   
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   (Page# 23-25) Methods: Cell culture and reagents. The experiments with Xenograft (including tumour measurements) were carried on in single blind.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
|     | • The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
|     | • A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
|     | • A statement indicating how many times each experiment was replicated |
|     | • The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
|     | • A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
|     | • The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
|     | • A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
|     | • Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.

Software
Policy information about availability of computer code

7. Software
Describe the software used to analyze the data in this study.

(Page 27, Page 29 and Supplementary Note Page 2) Method: Illumina GenomeStudio software and Genomics Suite 6.4 were used in 'Gene Expression Analysis'. Metaboanalyst was used in 'Targeted mass spectrometry analysis' and 'Lipidomics analysis on prostate cancer cell line'.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents
Policy information about availability of materials

8. Materials availability
Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No.

9. Antibodies
Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

(Page #25-26) Methods: Real-time PCR, western blotting and immunohistochemistry.

10. Eukaryotic cell lines
a. State the source of each eukaryotic cell line used.

(Page #23-25) Methods: Cell culture and reagents

b. Describe the method of cell line authentication used.

Yes, the procedure is described by the supplier (ATCC)

c. Report whether the cell lines were tested for mycoplasma contamination.

Yes, (Page #23-25) Methods: Cell culture and reagents. Mycoplasma is tested on monthly basis in the lab as standard procedure.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
Provide details on animals and/or animal-derived materials used in the study.

(Page # 23) Methods: Animals.

Policy information about studies involving human research participants

12. Description of human research participants
Describe the covariate-relevant population characteristics of the human research participants.

(Page # 25-26) Methods: Real-time PCR, western blotting, and immunohistochemistry. Prostate disease spectrum tissue array.