Therapeutic Targeting of the Warburg Effect in Pancreatic Cancer Relies on an Absence of p53 Function

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Abstract

The ‘Warburg effect’ describes a peculiar metabolic feature of many solid tumors, namely their increased glucose uptake and high glycolytic rates, which allow cancer cells to accumulate building blocks for the biosynthesis of macromolecules. During aerobic glycolysis, pyruvate is preferentially metabolized to lactate by the enzyme lactate dehydrogenase-A (LDH-A), suggesting a possible vulnerability at this target for small-molecule inhibition in cancer cells. In this study, we used FX11, a small-molecule inhibitor of LDH-A, to investigate this possible vulnerability in a panel of 15 patient-derived mouse xenograft (PDX) models of pancreatic cancer. Unexpectedly, the p53 status of the PDX tumor determined the response to FX11. Tumors harboring wild-type (WT) TP53 were resistant to FX11, a small-molecule inhibitor of LDH-A, to investigate this possible vulnerability in a panel of 15 patient-derived mouse xenograft (PDX) models of pancreatic cancer. Unexpectedly, the p53 status of the PDX tumor determined the response to FX11. Tumors harboring wild-type (WT) TP53 were resistant to FX11. [18F]-FDG PET-CT scans revealed a relative increase in glucose uptake in mutant TP53 versus WT TP53 tumors, with FX11 administration down-regulating metabolic activity only in mutant TP53 tumors. Through a noninvasive quantitative assessment of lactate production, as determined by 13C magnetic resonance spectroscopy (MRS) of hyperpolarized pyruvate, we confirmed that FX11 administration inhibited pyruvate-to-lactate conversion only in mutant TP53 tumors, a feature associated with reduced expression of the TP53 target gene TIGAR, which is known to regulate glycolysis. Taken together, our findings highlight p53 status in pancreatic cancer as a biomarker to predict sensitivity to LDH-A inhibition, with regard to both real-time noninvasive imaging by 13C MRS as well as therapeutic response. Cancer Res; 75(16): 1–10. ©2015 AACR.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth most common cause of cancer-related mortality in the United States, with an alarming rise in incidence and a projection that it will become the second most common cause of cancer deaths by 2030 (1). The 5-year survival rate of patients with advanced PDAC is ~5%, which remained largely unchanged over the last four decades. The current treatment options for advanced PDAC include the multidrug regimen FOLFIRINOX, or the combination of nab-paclitaxel plus gemcitabine (2). However, neither therapy is curative, providing a median survival of 11 and 8.5 months, respectively. The dismal prognosis of PDAC patients underscores the urgent need to identify novel therapeutic targets that exploit the underlying mechanistic vulnerabilities in cancer cells.

Reprogramming of cellular metabolism is one of the hallmarks of cancer (3). A critical aberration in metabolism that occurs in cancer cells is reflected in altered glucose metabolism. In normal tissues, lactate generation through glycolysis, and the resulting inefficient ATP production, is limited to anaerobic conditions, while pyruvate typically feeds into mitochondrial oxidative phosphorylation (OXPHOS) when adequate oxygen levels are present. In contrast, cancer cells preferentially convert glucose into lactate through glycolysis, even under normal oxygen concentrations, a phenomenon termed “aerobic glycolysis” or the Warburg effect (4, 5). The enzyme lactate dehydrogenase-A (LDH-A), the rate-limiting enzyme in the conversion of pyruvate into lactate, is overexpressed in many tumors, thereby providing a metabolic fuel for the production of building blocks for the biosynthesis of macromolecules needed for rapid proliferation and survival of cancer cells (6).

The “Warburg effect” describes a peculiar metabolic feature of many solid tumors, namely their increased glucose uptake and high glycolytic rates, which allow cancer cells to accumulate building blocks for the biosynthesis of macromolecules. During aerobic glycolysis, pyruvate is preferentially metabolized to lactate by the enzyme lactate dehydrogenase-A (LDH-A), suggesting a possible vulnerability at this target for small-molecule inhibition in cancer cells. In this study, we used FX11, a small-molecule inhibitor of LDH-A, to investigate this possible vulnerability in a panel of 15 patient-derived mouse xenograft (PDX) models of pancreatic cancer. Unexpectedly, the p53 status of the PDX tumor determined the response to FX11. Tumors harboring wild-type (WT) TP53 were resistant to FX11. [18F]-FDG PET-CT scans revealed a relative increase in glucose uptake in mutant TP53 versus WT TP53 tumors, with FX11 administration down-regulating metabolic activity only in mutant TP53 tumors. Through a noninvasive quantitative assessment of lactate production, as determined by 13C magnetic resonance spectroscopy (MRS) of hyperpolarized pyruvate, we confirmed that FX11 administration inhibited pyruvate-to-lactate conversion only in mutant TP53 tumors, a feature associated with reduced expression of the TP53 target gene TIGAR, which is known to regulate glycolysis. Taken together, our findings highlight p53 status in pancreatic cancer as a biomarker to predict sensitivity to LDH-A inhibition, with regard to both real-time noninvasive imaging by 13C MRS as well as therapeutic response. Cancer Res; 75(16): 1–10. ©2015 AACR.
dehydrogenase-A (LDH-A) is involved in the conversion of pyruvate into lactate, utilizing NADH as a cofactor. By converting pyruvate to lactate, LDH-A regenerates the NAD+ needed to maintain glycolysis and diverts pyruvate from being converted to acetyl-CoA for oxidative phosphorylation (6). Aerobic glycolysis provides bioenergetic intermediates and generates ATP, while simultaneously suppressing excessive reactive oxygen species (ROS) production. The lactate produced by cancer cells acidsifies the extracellular microenvironment, promoting invasion and metastases, reduced drug efficacy through ion tapping, and evading immune recognition (7–9). The increase in glycolytic flux is a metabolic strategy of tumor cells to ensure survival and growth in nutrient-deprived environments (10).

LDH-A is upregulated by various oncogenic transcription factors, such as hypoxia-inducible factor-1α (HIF1α) and c-Myc, in cancers (11). Conversely, it has been documented that reduction of fermentative glycolysis through LDH-A blockade results in the inhibition of tumor growth and metastases in various preclinical models, implicating LDH-A as a viable therapeutic target (12–17). Blockade of LDH-A activity with the pharmacologic inhibitor FX11 attenuates tumor progression across various preclinical models, including in PDAC cell lines (18). Given the expanding portfolio of pharmacologic inhibitors that target aberrant cancer metabolism (19, 20), it is imperative that molecular determinants of sensitivity and resistance to these inhibitors be identified, and further, clinically feasible assays that can provide insights into in vivo response in real-time be developed. In this study, we demonstrate that PDAC tumors are responsive to FX11 treatment in a TP53-dependent manner. We further demonstrate that 13C magnetic resonance spectroscopy (MRS) using hyperpolarized pyruvate provides a biochemically specific and clinically feasible approach for predicting in vivo response to LDH-A inhibition.

Materials and Methods

Patient-derived PDAC xenografts

All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University (Baltimore, MD) and the University of South Florida (Tampa, FL). Male nu/nu athymic mice (Harlan) were used for the study. Animals were maintained under pathogen-free conditions and a 12-hour light/12-hour dark cycle. Fresh PDAC specimens resected from patients at the time of surgery were implanted subcutaneously (s.c.) into the flanks of 6-week-old male athymic mice (Harlan). When cohorts of tumors reached approximately 200 mm³, mice (5 mice/group; 8–10 tumors in each arm) were randomly assigned to (i) control (vehicle) and (ii) FX11 (2.2 mg/kg), injected intraperitoneally, once daily for 4 weeks. Tumors were measured twice per week using a digital caliper, allowing discrimination of size modifications >0.1 mm. Individual tumor volumes were calculated using the formula: tumor volume $V = a \times b^2/2$, where $a$ being the largest dimension of the tumor, $b$ the smallest.

Effect of FX11 treatment on tumor cell proliferation and apoptosis

Excised tumors from the vehicle and FX11 treatment (harvested from the 28 day efficacy study) were fixed in 10% neutral-buffered formalin and processed into paraffin blocks. Sections were deparaffinized in xylene and rehydrated in graded-alcohol washes. Hematoxylin and cosin (H&E) staining was performed using standard procedure. The assessment of cellular proliferation was conducted using an anti-MIB-1 (Ki-67) antibody (clone K2, dilution 1:100, Ventana Medical Systems). IHC detection of apoptosis was performed using Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay with a commercial apoptosis detection kit (DeadEnd Fluorometric TUNEL System; Promega), according to the recommendations of the manufacturer. Stained sections were examined under light microscope and images were captured. Histograms for TUNEL and Ki-67 staining were generated by evaluating five high-power fields (hpf) of tumor section from two independent tumors per treatment arms (24).

TP53-induced glycolysis and apoptosis regulator and p53 IHC

Anti-TP53–induced glycolysis and apoptosis regulator (TIGAR) and anti-p53 antibodies (rabbit polyclonal to TIGAR, abcam, ab37910 and rabbit polyclonal to p53, abcam, ab4060) were used for TIGAR and p53 IHC, respectively. The staining was performed as per manufacturer’s protocol. Briefly, formalin-fixed, paraffin-embedded sections were deparaffinized and subjected to heat-mediated antigen retrieval in citrate buffer before blocking in 10% serum for 1 hour at room temperature. The primary antibodies were diluted at 1/400 (TIGAR) and 1/100 (p53). The sections were incubated with the sample for 1 hour at room temperature for TIGAR and 12 hours at 4°C for p53. A biotinylated goat anti-rabbit antibody was used as the secondary antibody (25, 26). Stained sections were examined microscopically at ×40 magnification and images were captured. The neoplastic areas were examined for staining and were graded according to the prevalence of both nuclear and cytoplasmic staining within the tumor. We used a 0 to 3 scale for staining intensity: 0, completely negative; 1, weak staining; 2, moderate staining; 3, strong staining (27). The staining was scored by evaluating sections from two separate tumors each from P420, JH033, P286, JH024, P253, P410, P194, and JH015.

Expression of human TIGAR transcripts in PDAC xenografts

Baseline TIGAR expression was determined in frozen tumors of two xenografts each with TP53 wild-type (WT) and TP53-mutant status. Total RNA was extracted using RNeasy Mini Kit (Qiagen), cDNA was synthesized with SuperScript First Strand System (Invitrogen), and qRT-PCR for huTIGAR expression was conducted using an anti-MIB-1 (Ki-67) antibody (clone K2, dilution 1:100, Ventana Medical Systems). IHC detection of apoptosis was performed using Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay with a commercial apoptosis detection kit (DeadEnd Fluorometric TUNEL System; Promega), according to the recommendations of the manufacturer. Stained sections were examined under light microscope and images were captured. Histograms for TUNEL and Ki-67 staining were generated by evaluating five high-power fields (hpf) of tumor section from two independent tumors per treatment arms (24).

In vivo efficacy of the LDH-A antagonist FX11 in patient-derived PDAC xenografts

Fifteen individual patient-derived PDAC xenografts were used for the study. The mutational status of common driver genes in PDAC has been previously described for these PDxs (23). Briefly, subcutaneously established tumors were harvested at the exponential growth phase, and were cut aseptically into cubes of 1 to 2 mm³. The tumor pieces were dipped in Matrigel and implanted on both flanks of 6-week-old male nu/nu athymic mice (Harlan). When cohorts of tumors reached approximately 200 mm³, mice (5 mice/group; 8–10 tumors in each arm) were randomly assigned to (i) control (vehicle) and (ii) FX11 (2.2 mg/kg), injected intraperitoneally, once daily for 4 weeks. Tumors were measured twice per week using a digital caliper, allowing discrimination of size modifications >0.1 mm. Individual tumor volumes were calculated using the formula: tumor volume $V = a \times b^2/2$, where $a$ being the largest dimension of the tumor, $b$ the smallest.
conducted using FAST SYBR Green Master Mix (Applied Biosystems) on a Step One Plus Real-Time PCR System (Applied Biosystems). Human PGK1 and murine β-actin were used as housekeeping genes. The primer sequences used for huTIGAR are Forward 5'-ATGGAATTTTGGAGAA-3' Reverse 5'-CCATGGCCCTCAGCTCAC-3' (28). Relative expression of the mRNA was estimated using the 2−ΔΔCT method.

FDG-PET imaging of PDAC xenografts

[18F]-FDG-PET imaging was used to determine the baseline glucose uptake and the impact of FX11 therapy. Tumor-bearing mice (N = 5 each) from two PDX models - JH024 (TP53 WT) and JH015 (TP53 mutant) were used for the study. Tumor-bearing mice in JH024 and JH015 PDXs were treated with vehicle or FX11 (2.2 mg/Kg) i.p. for 7 consecutive days. Mice were imaged for determining the tumor glucose uptake by [18F]-FDG-PET before the commencement of vehicle or FX11 treatment (D1) and on day 7 of vehicle or FX11 treatment (D7). On the day of imaging, each mouse was injected with 250 μCi of [18F]-FDG via the tail vein and imaged 45 minutes postinjection using the Mosaic HP (Philips) Small Animal PET imagers with 15 minutes static acquisition. PET images were

Figure 1.
In vivo antitumor effects of LDH-A inhibitor FX11 in human pancreatic cancer xenografts. A, FX11 treatment delays tumor progression, selectively in TP53-mutant (MUT) tumors. Tumors from fifteen individual patient-derived pancreatic cancer xenografts were implanted subcutaneously in athymic mice. Animals with established tumors (~200 mm³) were injected with vehicle or FX11 (2.2 mg/kg) i.p. for 4 weeks. Relative TGI was compared with vehicle-treated mice on day 28. Tumors with TP53 WT tumors were completely resistant to glycolytic inhibition, while a range of TGI was observed with mutant TP53 PDXs. B, three PDXs (P194, JH015, and P253) showed significant delay in tumor progression compared with vehicle-treated mice. Points, mean ± SEM. N = 8–10 tumors per group.

* P = 0.0478; ** P = 0.0011; *** P = 0.0002 compared with vehicle-treated mice.
reconstructed and coregistered with CT images using Amira version 5.2.2 (Visage Imaging; ref. 29).

Pyruvate sample preparation and animal handling

Samples (~30 μL) of [1-13C] pyruvic acid (Isotec) containing 15 mmol/L of OX63 trityl radical and 1.5 mmol/L of the gadolinium chelate dotarem were polarized at 3.35T and 1.4K in the HyperSense DNP polarizer (Oxford Instruments) for one hour. The hyperpolarized pyruvate sample was rapidly dissolved in 4 mL of a superheated alkaline buffer comprising 100 mg/L ethylenediaminetetraacetic acid, 40 mmol/L of TRIS buffer, 40 mmol/L of NaOH, and 30 mmol/L of NaCl. This produces 80 mmol/L concentration of pyruvate with physiologic pH (7.4) for administration to animals. Hyperpolarized [1-13C] pyruvate solution of 350 μL was intravenously injected over a period of 12 to 15 seconds through a catheter placed in the jugular vein of the tumor-bearing mice.

Representative PDXs that were sensitive (JH015, P253) and resistant (JH024, JH033) to FX11 treatment were chosen for the hyperpolarized13C-MRS study and T2-weighted MRI measurements. Mice with 500 mm3 subcutaneous tumors were selected to facilitate polarized substrate injections. The mice were treated with vehicle or FX11 (2.2 mg/kg) i.p. for 7 consecutive days (N = 4 mice/group).

**In vivo** 13C MR spectroscopy

As preparation for the MRI experiment, mice were infused with isoflurane in a plastic anesthesia chamber with scavenging. Anesthetized mice were placed in a mouse-specific holder within the MRI coil, outfitted with a mouse-specific nose cone inhalant anesthesia and scavenging system for imaging. This system also contains a pad for respiration monitoring, and endo-rectal fiber optic temperature monitoring device, a heated pad for maintaining core body temperature and leads for electrocardiography (ECG monitoring). In vivo MR experiments were performed on a 7T, 31-cm horizontal bore magnet (Agilent). Multi-slice T2-weighted anatomic images were acquired using a respiratory-gated spin-echo sequence with a TR = 60 ms, TE = 4000 ms, fat saturation, FOV 40 × 40 mm, echo train length = 8, matrix 256 × 128, slice thickness = 1 mm, 15 slices. Dynamic 13C MR spectra were acquired utilizing a dual tuned 1H-13C volume coil (M2M, 35 mm). All spectra were acquired using slice-selective pulse with a nominal flip angle of 9°, TR = 1 second, 30,000 complex points, and spectral width of 50 kHz. In vivo data acquisition started right before the pyruvate injection and collected single transient spectra over a period of 150 seconds from a 5-mm-thick tumor slice. The peak heights of pyruvate and lactate resonance spectra were used to calculate relevant ratios (Lac/Pyr).

![Figure 2](image-url)

**Figure 2.**

FX11 treatment induces apoptosis and inhibits tumor cell proliferation selectively in tumors with mutant TP53 status. IHC staining of TUNEL and Ki-67 in paraffin-embedded pancreatic tumors. Tumors resected on day 28 from vehicle and FX11 treatment groups (JH033, JH024, P253, and JH015) were used for analysis. Formalin-fixed, paraffin-embedded sections (3 μm) were stained for nuclear Ki-67 and TUNEL-positive tumor cells. A, representative photomicrographs (~20) from TP53 WT tumors (JH033 and JH024). No significant induction of apoptosis or inhibition of Ki-67 was seen in FX11-treated tumors compared with vehicle treatment. B, representative photomicrographs (~20) from mutant TP53 tumors (P253 and JH015) showing that FX11 treatment significantly induced apoptosis and inhibited tumor cell proliferation compared with vehicle treatment. Quantification of TUNEL and Ki-67-positive tumor cells is shown in the right panel of figures. The data representative of mean ± SD were generated by evaluating five different hpf from three different tumors per group. **P < 0.01 and ***P < 0.005 compared with vehicle-treated mice.
Statistical analysis

Statistical analysis was carried out by a two-tailed unpaired t test using GraphPad Prism 5 software. SEM or SD is either represented in the graphs or following the means of all measures, as stated in figure legends. Statistical significance was considered at the \( P < 0.05 \) level.

Results

Pharmacologic LDH-A inhibition leads to tumor growth inhibition selectively in TP53-mutant PDAC xenografts

We sought to determine the treatment effect of FX11, a small molecule that can inhibit LDH-A, in a panel of 15 pancreatic cancer PDXs. A wide range of responses to 4 weeks of FX11 therapy was observed in these PDXs, with tumor growth inhibition (TGI) ranging from approximately 40% to a few PDXs reaching a marginally larger volume than the control group (Fig. 1A). Overall, three of the PDXs had significant TGI with FX11 monotherapy (P253, JH015, and P194; Fig. 1B). In this panel, only two PDXs were KRAS WT (P410 and P420), and no specific pattern of response to FX11 was noted based on KRAS mutational status. Similarly, SMAD4 mutational status did not yield any discernible pattern of response (Supplementary Table S1). On contrary, when the waterfall plot was segregated based on TP53 status, nearly all PDXs with any evident TGI (including the three tumors with significant inhibition) were TP53 mutant, while PDXs with minimal response or marginal enhancement in growth were uniformly TP53 WT. We assessed proliferation (by Ki-67 nuclear labeling) and apoptosis (using TUNEL staining) in two TP53 WT (JH033 and JH024) and two TP53 mutant (P253 and JH015) PDXs, and identified a significant reduction in proliferation, and a significant increase in apoptosis post-FX11 therapy that was restricted to the TP53-mutant xenografts (Fig. 2A and B). Overall, these findings suggested that TP53 status is likely to be a key molecular determinant of response to pharmacologic LDH-A inhibition.

We also measured the expression of the p53 target TIGAR, whose expression has been linked to decreased glycolytic conversion of pyruvate to lactate. Using IHC, we found that TIGAR expression was positively correlated with TP53 WT status, and its expression appeared diminished in TP53-mutant tumors (Fig. 3A and B). Quantification of the p53 and TIGAR staining intensity in tumor sections of TP53 WT PDXs revealed a significant increase in p53 and TIGAR expression compared with TP53-mutant PDXs (Fig. 3C). Baseline TIGAR mRNA expression was also reduced in TP53-mutant tumors compared with tumors with TP53 WT tumors, with a significant downregulation in one of two PDXs (Supplementary Fig. S1).

FDG-PET imaging of PDAC xenografts

In light of the differential responses to LDH-A inhibition, we interrogated the pattern of 18F-deoxy-glucose (FDG) uptake at baseline and following FX11 therapy in two PDXs, one with
WT TP53 (JH024) and the second with a TP53 mutant (JH015). The FDG uptake rates varied between JH024 and JH015 tumors. At baseline (D1), JH015 tumors had elevated FDG uptake compared with JH024, indicating greater reliance on glycolysis (Fig. 4A and B). Notably, there were no alterations in tumor FDG uptake following 7 days of FX11 therapy in JH024 (Fig. 4A). In contrast, the avid glucose uptake observed in JH015 xenografts at baseline was significantly reduced following 7 days of FX11 therapy (Fig. 4B).

Hyperpolarized 13C MR spectroscopy confirms that changes in lactate flux upon FX11 therapy are restricted to TP53-mutant xenografts

The recent development of hyperpolarized MR spectroscopic imaging methods to probe the biochemical and metabolic profile of tumors using 13C-labeled pyruvate as the tracer, and then monitor its intracellular conversion to various metabolites, offers a unique opportunity to examine in vivo responses to metabolism-targeted therapies. In order to assess the metabolic conversion of hyperpolarized pyruvate to lactate, two sets of PDXs that were either sensitive to FX11 treatment (JH015 and P253, both TP53 mutant) or resistant to FX11 treatment (JH024 and JH033, both TP53 WT), respectively, were chosen for hyperpolarized 13C-MRS study.

Representative dynamic 13C MRS spectra of JH033 tumor (FX11 resistant) captured before FX11 treatment and 7 days FX11 treatment are shown in Fig. 5A and B, respectively. Pyruvate and lactate peak intensities over time are shown in Fig. 5E and F. FX11 treatment was ineffective in altering the conversion flux from pyruvate to lactate in JH033. In contrast with the JH033 spectra, dynamic 13C MRS spectra of P253 showed that pyruvate-to-lactate conversion was significantly reduced after 7 days of FX11 treatment as compared with spectra captured before FX11 treatment (Fig. 5C and D), and this was confirmed in the pyruvate and lactate peak intensities over time (Fig. 5G and H). Seven days vehicle treatment did not alter the flux of hyperpolarized pyruvate to lactate conversion in both JH033 and P253 tumors (13C MRS spectra not shown).

The integrated lactate-to-pyruvate ratio (Lac/Pyr) can be used to represent a drug therapy response marker in this study. The Lac/Pyr flux ratio was calculated from the area under the curve (as regarded a “Model-Free” approach) of the metabolic flux from the dynamic scan (30). The Lac/Pyr flux ratios in response to FX11 treatments were significantly different between tumors sensitive
(P253 and JH015) and resistant (JH024 and JH033) to FX11 treatment (Fig. 6A). The Lac/Pyr ratios increased with time in the resistant (JH024 and JH033) tumors (Fig. 6A). In stark contrast, the Lac/Pyr of both sensitive tumors (P253 and JH015) showed a striking decrease in response to FX11 treatment (Fig. 6A). The tumor growth curves of representative tumors resistant (JH033) and sensitive (P253) to FX11 treatment are shown in Fig. 6B and C. We also evaluated the unidirectional conversion rate constants (k_p = pyruvate-to-lactate) using modified Bloch’s equation of kinetic model (31). Consistent with the above results, the conversion rate constantly (k_p) decreased in FX11-sensitive tumors and increased in FX11-resistant tumors (Table 1).

Discussion

Persistent aerobic glycolysis is a key metabolic dependency in tumorigenesis. LDH-A is a key regulator of glycolysis and plays a critical role in tumor maintenance (6). LDH-A is frequently upregulated in aggressive cancers, and expression levels frequently correlate with poor prognosis (32, 33). Furthermore, knockdown of LDHA compromises the tumorigenic potential of malignant cells (6). LDH-A expression is significantly elevated in pancreatic cancer compared with paired normal tissues (12, 34). Although forced expression of LDH-A enhances the proliferation of pancreatic cancer cells, knocking down of LDHA transcript expression inhibited cell growth and induced apoptosis (12). Silencing LDHA expression also inhibited growth of pancreatic cancer cells in vivo, suggesting LDH-A a potential target for pancreatic cancer therapy (12). Of note, systemic inhibition of LDH-A may not produce side effects in humans, since hereditary LDH-A deficiency does not provoke any symptoms under baseline circumstances (35).

The advent of metabolism-targeted agents has garnered interest in identifying genetic determinants of responses to these agents in cancer cells, especially for therapies that are not geared directly against specific activating events like IDH1 mutations. In this study, we examined the therapeutic efficacy of a small-molecule LDH-A inhibitor across a panel of PDAC PDXs. Human pancreatic cancer xenografts used in the present study are annexed with desmoplastic stroma (Supplementary Fig. S2), a well-characterized feature of primary tumors and metastatic lesions of human PDAC (36). Masson’s trichrome staining and H&E histology from 2D and 3D cultures of PDXs showed that tumors were enriched with connective tissue, irrespective of their TP53 status (Supplementary Fig. S2). We identified that the sensitivity to LDH-A inhibition is dependent on p53 status of the tumor, TP53-mutant tumors demonstrating sensitivity, and TP53 WT tumors demonstrating refractoriness to FX11 treatment. The basis for resistance to glycolytic inhibition could be the reduced dependence on glucose as an elemental fuel, as TP53 wild-type PDXs had minimal FDG uptake. Of note, expression of TIGAR was higher in PDXs with WT TP53 function, whereas TP53-mutant tumors demonstrated decreased TIGAR expression (37–39). In TP53 WT cells, TIGAR expression functions to decrease the levels of fructose-2,6-bisphosphate, which
suppresses glycolysis by diverting glucose-6-phosphate into the pentose phosphate pathway. The correlation of TIGAR expression with p53 status in pancreatic cancer PDXs could explain one potential mechanism for the reduced sensitivity to LDH-A inhibition in p53 WT, high TIGAR cases, by reducing glycolytic dependence.

The understanding of cancer metabolism has deep roots in molecular biology and genetics but the ability to detect metabolism as it occurs in tumors without a biopsy is largely limited to PET imaging of enhanced glucose uptake into tumors using $^{18}$F-fluorodeoxyglucose. The recently discovered hyperpolarized MRI using dynamic nuclear polarization technology has the potential of providing real-time images of metabolic processes in tumors at unprecedented levels of sensitivity for monitoring therapy responses (40–43). Hyperpolarized $^{13}$C magnetic resonance provides a unique ability to noninvasively assess metabolic fluxes and downstream product(s) in real time, which has been utilized for tumor diagnosis, to stage the extent of disease, and to monitor the response to therapy.

Transmembrane transport of pyruvate, lactate, and ketone bodies is mediated by monocarboxylate transporters -1 and 4 (MCT-1 and MCT-4; ref. 44). It has been documented that lactate produced by tumor cells can be taken up by stromal cells to regenerate pyruvate that either can be extruded to refuel the cancer cell or can be used for oxidative phosphorylation (45). HIF1α stimulates the conversion of glucose to pyruvate and lactate by upregulating glucose transporter isoform 1 (GLUT1), hexokinase, and LDH-A, as well as the lactate-extruding enzyme MCT-4 (46). A recent $^{13}$C MRS study confirmed the modulatory effect of LDH-A, lactate, and MCT isoforms in hyperpolarized pyruvate to hyperpolarized lactate conversion in cancer cell lines (47). Results obtained in our $^{13}$C MRS experiments demonstrated a decrease in pyruvate to lactate conversion following FX11 treatment, in JH015 and P253, both TP53-mutant PDXs. In order

Figure 6. FX11 treatment reduces the lactate/pyruvate ratio, selectively in PDXs sensitive to FX11 treatment. A, Lac/Pyr ratio was reduced with FX11 treatment in PDXs sensitive to FX11 (P253 and JH015). However, the ratio was increased in PDXs resistant to FX11 treatment (JH024 and JH033). B and C, tumor growth curves representative of tumors that were resistant (JH033) and sensitive (P253) to FX11 treatment. $N = 8–10$ tumors in each group. Errors bars represent standard error of mean ± SEM. ** $P = 0.0011$ compared with vehicle-treated mice.
to characterize the contribution of tumor lactate pool, LDH-A, HIF-1α, and MCT isoforms (MCT-1 and MCT-4) in attenuating hyperpolarized pyruvate to lactate production, we conducted baseline gene expression analysis and measuring the tumor lactate metabolism during the same animal enabled us to measure the flux changes and differential kinetics in pyruvate-to-lactate conversion within an individual tumor over time. Of note, we were able to gauge differences in pharmacodynamics of pyruvate to lactate conversion in real time at a point even before reduction in tumor volumes was observed in FX11-sensitive tumors. Our studies demonstrated the feasibility of using 13C hyperpolarized metabolic imaging as a noninvasive biomarker for monitoring response to metabolic therapy. The capability of MRS to noninvasively obtain metabolic information is a valuable asset in the clinic and can provide molecular signatures of specific biologic processes as a complementary imaging modality of FDG-PET.

The small number of fifteen xenografts studied and the lack of detailed examination of factors that might affect dependence on glycolytic metabolism, including but not limited to intratumoral pH, O2 tension, interstitial pressure, tumor size, and growth fraction, are limitations to this study. Even though we have not conducted in vitro studies in support of the impact of mutant p53 in targeting Warburg effect, previous report indicated that tumor-associated mutant p53 stimulates the Warburg effect in cultured cells and demonstrated that targeting altered glucose metabolism could be a feasible therapeutic strategy for tumor carrying mutant p53 (48). Additional studies are needed to fully understand the mechanistic link in supportive of the influence of mutant p53 in deciding the sensitivity of LDH-A-targeted therapy, or whether the phenomenon is demonstrable only in the in vivo milieu. Our data show that pharmacologic inhibition of LDH-A may have utility in the treatment of tumors by reducing tumor growth of TP53-mutant tumors. In summary, we observe that FX11, a LDH-A small-molecule antagonist, attenuated in vivo tumor growth, induced apoptosis and reduced tumor cell proliferation, specifically in pancreatic cancer PDXs with mutant TP53. Analysis of FDG PET-CT scans of animals demonstrate that PDXs with mutant TP53 tumors have relatively high glucose uptake compared with tumors with TP53 WT status. In addition, using hyperpolarized 13C magnetic resonance spectroscopy, we demonstrate that FX11 treatment inhibited the pyruvate to lactate conversion only in tumors with mutant TP53 status. Currently, we do not understand the mechanistic basis for the resistance of WT TP53 pancreatic cancer PDXs to FX11, particularly regarding the lack of an effect on the in vivo conversion of pyruvate to lactate. Furthermore, it is notable that high concentrations of FX11 could have off-target effects beyond inhibition of LDH-A. Nonetheless, our collective studies are the first to document a link between tumor p53 status and LDH-A inhibitor sensitivity. Our work also suggests that the elevated glucose metabolism in TP53-mutant tumors can be exploited for the preferential targeting of these tumors by LDH-A inhibitor therapy. The effectiveness of the therapy can be monitored by in vivo metabolic imaging and can be readily applied to humans for monitoring tumor response to therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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Table 1. Apparent rate constants kP (pyruvate-to-lactate conversion) of tumors from FX11 pretreatment and D7 FX11 treatment

<table>
<thead>
<tr>
<th>PDXs</th>
<th>Prior to FX11 treatment</th>
<th>D7 FX11 treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P253</td>
<td>0.054 ± 0.004</td>
<td>0.029 ± 0.005</td>
<td>0.011</td>
</tr>
<tr>
<td>JH015</td>
<td>0.055 ± 0.003</td>
<td>0.033 ± 0.004</td>
<td>0.013</td>
</tr>
<tr>
<td>JH024</td>
<td>0.059 ± 0.005</td>
<td>0.077 ± 0.004</td>
<td>0.019</td>
</tr>
<tr>
<td>JH033</td>
<td>0.061 ± 0.004</td>
<td>0.083 ± 0.003</td>
<td>0.016</td>
</tr>
</tbody>
</table>

NOTE: N = 4 tumors in each xenografts; mean ± SD; P < 0.05, Student t test.
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