Expression and role of PAICS, a *de novo* purine biosynthetic gene in prostate cancer

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BACKGROUND. Our goal was to investigate *de novo* purine biosynthetic gene PAICS expression and evaluate its role in prostate cancer progression.

METHODS. Next-generation sequencing, qRTPCR and immunoblot analysis revealed an elevated expression of a *de novo* purine biosynthetic gene, Phosphoribosylaminoimidazole Carboxylase, Phosphoribosylaminoimidazole Succinocarboxamide Synthetase (*PAICS*) in a progressive manner in prostate cancer. Functional analyses were performed using prostate cancer cell lines- DU145, PC3, LnCaP and VCaP. The oncogenic properties of PAICS were studied both by transient and stable knockdown strategies, *in vivo* chicken chorioallantoic membrane (CAM) and murine xenograft models. Effect of BET bromodomain inhibitor JQ1 on the expression level of PAICS was also studied.

RESULTS. Molecular staging of prostate cancer is important factor in effective diagnosis, prognosis and therapy. In this study, we identified a *de novo* purine biosynthetic gene; *PAICS* is overexpressed in PCa and its expression correlated with disease aggressiveness. Through several *in vitro* and *in vivo* functional studies, we show that PAICS is necessary for proliferation and invasion in prostate cancer cells. We identified JQ1, a BET-domain inhibitor previously implicated in regulating *MYC* expression and demonstrated role in prostate cancer, abrogates *PAICS* expression in several prostate cancer cells. Furthermore, we observe loss of *MYC* occupancy on *PAICS* promoter in presence of JQ1.

CONCLUSIONS. Here, we report that evaluation of *PAICS* in prostate cancer progression and its role in prostate cancer cell proliferation and invasion and suggest it as a valid therapeutic target. We suggest JQ1, a BET-domain inhibitor, as possible therapeutic option in targeting *PAICS* in prostate cancer.

INTRODUCTION

Prostate cancer (PCa) is one of the most common cancers among men in the United States and across the world (1,2). Prostate organ function is critically dependent on steroid hormone androgen. Early stages of localized prostate cancer are usually androgen receptor (AR) dependent and therefore can be effectively treated with anti-androgens. However, over time PCa progresses into a castration resistant prostate cancer (CRPC) that can be either androgen dependent or independent. The CRPCs are genetically and molecularly heterogeneous, aggressive and metastatic PCa are lethal (3).

Besides AR, aberrant levels of *MYC* expression either as genetic dysregulation (amplification) or epigenetic modulation (increased expression) in PCa is associated with poor prognostication (4-6). However, *MYC* is a transcription factor that regulates myriad of metabolic pathways which includes but is not limited to purine biosynthesis, amino acid metabolism, glycolysis, etc (7,8). *MYC* targeting has been difficult endeavor and is considered "undruggable" (9). Alternate strategies to counter *MYC*-mediated oncogenesis include targeting synthetic lethal partners for *MYC* or pathways regulated directly or indirectly by increased *MYC* expression (10). *MYC* mediated regulation of purine biosynthetic pathway have recently been demonstrated in androgen sensitive prostate cancer cells (8).

Recently, there has been renewed appreciation of role of metabolism in cancer initiation, progression and metastasis (11). Numerous metabolic genes and metabolites have become candidates for diagnosis, prognosis and therapeutic targeting. For example, IDH1 and IDH2 mutations have been discovered to confer altered properties that results in production of α-hydroxyglutarate instead of α-ketoglutarate (12). This in turn triggers epigenetic changes that promote oncogenesis. Sarcosine accumulation during prostate cancer progression is potential diagnostic marker (13). Among other examples, amino acids such as glycine and glutamine

dependency of rapidly proliferating cells, cancer specific activation of isoform variants of hexokinase (hexokinase2) have therapeutic potential (14).

Our present study discovered an increased expression of *de novo* purine metabolic enzyme Phosphoribosylaminoimidazole Carboxylase, Phosphoribosylaminoimidazole Succino-carboxamide Synthetase, (PAICS*)* in prostate cancer. Our analyses show increased *PAICS* expression correlates with progression of prostate cancer from benign, localized to metastatic cancer thereby revealing its potential as progression marker. Through *in vitro* and *in vivo* studies, we demonstrate the necessary role of PAICS in prostate cancer cell growth, invasion and metastasis. We demonstrate that PAICS expression can be abrogated using JQ1, a BET-domain protein inhibitor, potentially through direct targeting of MYC protein. Our study reveals PAICS as a marker of disease progression. In addition, we show its essential role in tumor growth and metastasis that can be potentially targeted using BET bromodomain protein inhibitors.

MATERIALS AND METHODS

Cell Lines

Prostate cancer cell lines DU145, PC3 and LnCaP were grown in RPMI 1640 (Life Technologies, NY), while VCaP was grown in DMEM with penicillin-streptomycin (100 U/mL) and 10% FBS (Invitrogen) in 5% CO_2 cell culture incubator. Prostate cancer cells were infected with lentiviruses expressing PAICS shRNA or non-targeting shRNA controls and stable cell lines were generated by selection with 1 μg/ml puromycin (Life Technologies, NY).

Benign and Tumor Tissues

In this study, we utilized tissues from clinically localized prostate cancer patients who underwent radical prostatectomy. Samples were also obtained from androgen-independent metastatic prostate cancer patients from a rapid autopsy program through the University of Michigan Prostate SPORE Tissue Core as described previously(15,16). The detailed clinical and pathological data are maintained in a secure relational database. The Institutional Review Board at the University of Michigan Medical School approved this study. Both radical prostatectomy series and the rapid autopsy program are part of the University of Michigan Prostate SPORE Tissue Core.

Gene expression from The Cancer Genome Atlas (TCGA)

The patients clinical data for prostate adenocarcinoma (PRAD) were downloaded using TCGA-assembler (17). However, downloaded data comprised of only tumor pathologic (pT) and node pathologic (pN) information. Thus based on pT and pN data as per "https://cancerstaging.org/references-tools/quickreferences/Documents/ProstateSmall.pdf", samples were categorized into primary and metastatic tumor. Afterwards, level3 TCGA RNA-seq data (including raw_read_count and scaled_estimate for each sample) for all primary tumor, metastatic tumor and matched normal samples were downloaded using TCGA-assembler. Transcript per million values for each gene was obtained by multiplying scaled_estimate by 1,000,000. Boxplot was generated using R [https://cran.r-project.org/].

Immunohistochemistry

Benign and prostate cancer tissues were obtained from the radical prostatectomy series at the University of Michigan and from the Rapid Autopsy Program, both part of the University of Michigan Prostate SPORE programs, through appropriate informed consent. Institutional Review

Board approval was obtained to procure and analyze the tissues used in this study. Immunohistochemistry (IHC) was carried out to evaluate PAICS expression using mouse monoclonal antibody against PAICS (GeneTex, CA, Cat# GTX83950). IHC was performed using an automated protocol developed for the DISCOVERY XT automated slide staining system (Ventana Medical Systems, Inc.,) using Ultramap anti-mouse HRP (Cat# 760-4313,Ventana Medical Systems, Inc.,) and was detected using ChromoMap DAB (Cat#760-159, Ventana Medical Systems Inc.). Hematoxylin II (Cat#790-2208 Ventana-Roche, Tucson, AZ, USA) was used as the counterstain. The study pathologist Dr. Kunju (P.K.) evaluated IHC staining.

PAICS Progression Analysis (TMA 145)

TMA 145 contained 213 cores from 57 patients. After exclusion of lost, stromal, and necrotic tumor cores, 167 cores remained from 50 patients. After exclusion of HGPIN samples, 156 cores remained from 49 patients. Cores were only retained for subsequent analysis if their intended status (localized cancer vs. benign) agreed with the evaluation status, resulting in 149 cores from 49 patients. Product score (the product of staining percentage, 0-100%, and staining intensity, 0-4), was computed for each core, resulting in a measurement of staining ranging from 0 to 400. Multiple cores from the same patient were aggregated whenever they were of the same type (benign, localized cancer, or metastatic) by taking the median product score across cores for that patient. This resulted in 54 data points from 49 patients. The 5 patients with multiple measurements each contained both cancer and benign cores on the TMA.

Immunoblot Analyses

Antibodies used in the study are listed in **Table S1**. All antibodies were employed at dilutions optimized in our laboratory. For immunoblot analysis, 10 μg protein samples were separated on a SDS-PAGE and transferred onto Immobilon®-P PVDF membrane (EMD Millipore, Billerica, MA). The membrane was incubated for one hour in blocking buffer (Tris-buffered saline, 0.1% Tween [TBS-T], 5% nonfat dry milk) followed by incubation overnight at 4°C with the primary antibody. After two washes for five minutes each with TBS-T, the blot was incubated with horseradish peroxidase-conjugated secondary antibody (1:5000) for 1 h at room temperature and signals were visualized by Luminata™ Crescendo chemiluminescence western blotting substrate as per manufacturer's protocol (EMD Millipore, Billerica, MA).

RNA Interference and Transfection

The PAICS and non-targeting small interfering RNA (siRNA) duplexes (**Table S2**) were purchased from Dharmacon, Lafayette, CO (GE Healthcare, USA). Transfections were performed with Lipofectamine® RNAiMAX (Life Technologies, NY) reagent. PAICS shRNAs (**Table S2**) were purchased from SBI (System Biosciences, Mountain View, CA). Lenti-viruses for these stable knockdowns were generated by the University of Michigan Vector Core. For RNA interference, we seeded prostate cancer cells at 1×10^5 cells per well in a 6-well plate and twelve hours later the cells were transfected with siRNA duplexes. A second identical transfection was performed 24 hours later. Seventy-two hours after the first transfection, cells were harvested for RNA isolation or immunoblot analysis.

Treatment with JQ1

Prostate cancer cells – DU145, PC3, LnCaP and VCaP cells were seeded in 6-well plates and propagated at 37 °C in an atmosphere of 5 % CO_2 in a humidified incubator overnight. Cells

were then treated with 1 or 5 uM of JQ1 (Cat# 27400; BPS Bioscience, Inc., San Diego, CA). JQ1 was dissolved in dimethyl sulfoxide (DMSO; Thermo Fisher Scientific, Waltham, MA, USA).

Cell Proliferation Assays

Cell proliferation was measured by cell counting. For this, transient and stable PAICS knockdowns were used. After 72 h of transfection using specific siRNA, the cells were trypsinized and seeded at a density of 10,000 cells/well in 24-well plates (n = 3). Non-targeting si/sh-RNA-treated cells were served as controls. Then the cells were trypsinized and counted at specified time points by Z2 Coulter particle counter (Beckman Coulter, Brea, CA). Each experiment has been performed with three replicates per sample.

Cell cycle analyses

PC3 cells transfected with non-targeting siRNAs or PAICS siRNAs in 6-well plate were (1×10^5 cells/well) resuspended in 0.5ml Dulbecco's phosphate-buffered saline (DPBS). Drop-wise 100% cold ethanol was added to the cells. The cells were vortexed and incubated in Ethanol for 20 mins, before storing at 4 ^{o}C for further use. For staining, cells in alcohol were pelleted (2000rpm/5 min). The alcohol was decanted and cells were washed with DPBS twice. Then, the cells were resuspended in DPBS containing 50µg/ml propidium iodide and 100µg/ml RNase A. Cells were incubated in dark for 20 min before flow cytometry analyses. Each sample was measured in triplicate.

Wound Healing Assay

Prostate cancer cells- DU145, PC3 scramble shRNA or PAICS stable knock down cells were seeded in 6-well plates in RPMI-1640 containing 10% fetal bovine serum and puromycin (1 µg/ml), and then allowed to grow to confluent monolayer. The cells were serum starved for 12 h and replenished with 10% FBS-RPMI medium. The wound-induced migration was triggered by scraping the cells with a 200 ul pipette tip, washed with D-PBS and replenished with respective medium. The wound was imaged immediately (0 h) and at 12 h with an inverted phase-contrast microscope under 4X objective.

Matrigel Invasion Assay

Matrigel invasion assays were performed as described earlier (18-20). Various test cells were seeded onto Corning® BioCoat™ matrigel® matrix (Corning, New York) in the upper chamber of a 24-well culture plate. The lower chamber containing respective medium was supplemented with 10% serum as a chemo-attractant. After 48 h, the non-invading cells and matrigel matrix were gently removed with a cotton swab. Invasive cells located on the lower side of the chamber were stained with 0.2 % crystal violet in methanol, air-dried and photographed using an inverted microscope (4X). The invaded cells were quantified by colorimetric assay. For colorimetric assays, the inserts were treated with 150 µl of 10% acetic acid and the absorbance measured at 560 nm.

Colony Formation Assay

After 72 h of transfection, untreated, non-targeting and PAICS siRNA treated cells were counted and seeded 800 cells per one well of 6-well plates (triplicates) and incubated at 37 ^{o}C, 5% CO_2 for 10 days. Colonies were fixed with 10% (v/v) glutaraldehyde for 30 min and stained with crystal violet (St. Louis, MO USA) for 20 min. Then the photographs of the colonies were taken

using Amersham Imager 600RGB (GE Healthcare Life Sciences, PA, USA). Colony quantification was done using ImageQuant TL Colony v8.1 software (GE Healthcare Life Sciences, PA, USA).

Chromatin Immunoprecipitation (ChIP) Assays

ChIP assays were carried out with respective antibodies (**Table S1**) using the EZ-Magna ChIP kit (Millipore, Billerica, MA) as described.(19) The primer sequences for the promoters analyzed are provided in **Table S4**.

Chicken Chorioallantoic Membrane (CAM) Assay

The CAM assay for local cell invasion, intravasation, metastasis and tumor (or xenograft) formation was performed as previously described (18-22). After 3 days of implanting the cells in each egg, lower CAM was harvested and extra-embryonic tumors were isolated and weighed. For metastasis assay, the embryonic livers were harvested on day 18 of embryonic growth and analyzed for the presence of tumor cells by quantitative human Alu-specific PCR. Genomic DNA from lower CAM and livers were prepared using Puregene DNA purification system (Qiagen, Valencia, CA) and quantification of human-Alu was performed as described (18-22). An average of 8 eggs per group was used in each experiment.

Tumor Xenograft Model

All procedures involving mice were approved by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan and conform to all regulatory standards. To evaluate the role of PAICS in tumor formation *in vivo,* we propagated stable PAICS knockdown PC3 cells using two-independent shRNAs and non-targeting shRNA control cells, and inoculated

1×10^6 cells subcutaneously into the dorsal flank of 5-week-old male Athymic nude mice (n = 8 for each group; Harlan Laboratories, Evigo Indianapolis, IN). The tumor data obtained using scramble cells is same as used in an earlier study since PAICS tumor xenograft study was conducted *simultaneously* using common control animals (19). Tumor size was measured biweekly, and tumor volumes were calculated using the formula $(\pi/6)$ $(L \times W^2)$, where L = length and W = width of the tumor. After end of the experiment, mice from different groups were sacrificed; the tumors were then photographed, weighed and plotted.

Statistical Analysis

To determine significant differences between two groups, Student's two-tail t test was used for all experiments except for microarray, p-values <0.05 considered significant.

RESULTS

Through the analysis of publicly available prostate cancer gene expression profiling data, transcriptome sequencing data we establish overexpression of *de novo* purine biosynthetic enzyme PAICS in prostate cancer. Furthermore, immunohistochemistry with prostate tissue microarray demonstrate PAICS expression increases with disease progression with metastatic prostate cancer having highest expression. Through RNA interference studies we show that PAICS is necessary for prostate cancer cell growth, invasion and colony formation suggesting PAICS is required for cancer cells. We then demonstrate that PAICS is necessary for tumor growth and metastasis by xenograft studies in mouse and chorioallantoic membrane assay (CAM). Finally, we demonstrate that PAICS expression can be targeted using JQ1, an inhibitor of BET bromodomain proteins possibly through disruption of MYC-mediated PAICS regulation.

We therefore conclude that PAICS is potential biomarker for prostate cancer and amenable to therapeutic targeting using BET-domain inhibitors.

PAICS is a biomarker for prostate cancer progression

Our analysis of publicly available prostate cancer gene expression profiling data using Oncomine database [Oncomine™ Platform (Life Technologies, Ann Arbor, MI) (23) suggested significant overexpression *PAICS* in multiple independent gene expression profiling studies (**Fig. 1A**). Analysis of transcriptome sequencing of prostate cancer confirmed the increased PAICS expression in primary prostate cancer as well as metastatic prostate tumors (**Fig. 1B**). Moreover, TCGA data shows that PAICS is over-expressed in metastatic prostate adenocarcinoma (**Fig. 1C**). Next, we validated this observation by immunoblot analysis using specific antibody against PAICS, which showed a significantly increased PAICS protein in primary and metastatic PCa tissue lysates (**Fig. 1D**). Furthermore, we validated our observations through immunohistochemistry using a prostate tissue microarray containing multiple benign, primary PCa and metastatic PCa samples (**Fig. 1E**). We observed a strong cytoplasmic staining for PAICS in primary PCa as well as metastatic PCa. The staining intensity analysis indicated that PAICS is a progression associated marker with increasing expression observed from primary PCa and Metastatic PCa (**Fig. 1F**). The difference in PAICS product scores between benign and localized cancer patients is statistically significant ($P < 0.001$, Student's t-test), as is the difference between benign and metastatic tissues ($P < 0.001$).

PAICS is essential for prostate cancer cell proliferation and invasion

To determine the role of PAICS in prostate cancer cell growth, we transiently knocked down PAICS using two specific and independent siRNA duplexes in aggressive prostate cancer cell

lines DU145 and PC3 and conducted cell proliferation, invasion and colony formation experiments. Knockdown of PAICS was observed by immunoblot analysis (**Fig. 2A**). Simultaneously, the cell proliferation assays were conducted in DU145 and PC3 cells by counting the cells at various time intervals. PAICS knockdown reduced prostate cancer cell proliferation (**Fig. 2B**). PAICS knockdown reduced the number of colonies as compared to untreated and non-targeting siRNA treated cells as measured by colony formation assay (**Fig. 2C**). Furthermore, knockdown of PAICS reduced cancer cell invasion as measured by Boyden chamber matrigel invasion assay (**Fig. 2D**). Additionally, stable knockdowns of PAICS were achieved using two specific and independent shRNAs both in DU145 and PC3 cells (**Supplementary Fig. 1A**). Similar to transient knockdowns, stable knockdowns also reduced prostate cancer cell proliferation (**Supplementary Fig. S1A**). Moreover, knockdown cells displayed reduced cell motility as compared to non-targeting shRNA cells (**Supplementary Fig. S1B**). Cell cycle analysis in PAICS knockdown, showed decreased population in G1 and increased S-phase arrested cells consistent with PAICS role in purine biosynthesis (**Supplementary Fig. S2**). Thus, these *in vitro* experiments demonstrated the essential role of PAICS in prostate cancer cell proliferation, colony formation and invasion.

Role of PAICS in prostate cancer tumor growth

To substantiate the *in vitro* experiments, we took advantage of both *in vivo* CAM (chick chorioallantoic membrane) assay and mouse xenograft models. Earlier, CAM assays were used successfully as an *in vivo* model to investigate the tumorigenic potential, to assess cell intravasation and metastasis to distant organs (19-22). To test this, we generated two independent stable PAICS knockdowns in prostate cancer cells (**Fig. 3A**). These stable PAICS knockdown cells showed a significant reduction in tumor weight (**Fig. 3B**), as well as decreased intravasation

(**Fig. 3C**) and metastasis (**Fig. 3D**) to liver. We demonstrate that PAICS knockdown impairs ability of prostate cells to grow, invade and metastasize. Independently, we used mouse prostate xenograft model for tumor growth using athymic nude mice to check role of PAICS in tumor growth and progression. As observed in CAM assay, we saw significant reduction in tumor growth and weight of PAICS shRNA cells compared to control shRNA cells (**Fig. 3E** and **F**), demonstrating its *in vivo* crucial role in prostate tumor growth.

Bromodomain inhibitor JQ1 inhibits PAICS expression

Inhibitors of bromodomain and extra-terminal (BET) domain family of epigenetic reader proteins have been demonstrated in anti-tumor activity in various cancer models (24-27). Earlier it was demonstrated that BET inhibitor JQ1 reduces *MYC* expression in several cancers including multiple myeloma (28), prostate (24), liver(29), bladder (30), pancreatic ductal adenocarcinoma (27) and others. Moreover, recent studies showed inducible MYC mediated transcriptional activation of PAICS expression in androgen-dependent prostate cancer cells (7,8). To therapeutically identify the role of JQ1 in MYC-mediated PAICS expression, we treated androgen-independent DU145, PC3, and androgen-sensitive prostate cancer cell lines LnCaP and VCaP with 1 or 5 uM of JQ1 for 48 h. JQ1 treatment dramatically reduced both MYC and PAICS expression both at RNA (**Supplementary Fig. S3**) and protein (**Fig. 4A**) levels. Next, we sought to conduct chromatin immunoprecipitation (ChIP) using MYC-specific antibody and followed by qRTPCR using both DMSO and JQ1 treated ChIP-DNA. QRTPCR analysis using several *PAICS*-promoter specific primers, we demonstrate that JQ1 abolished MYC binding at the *PAICS* promoter. Thus we conclude JQ1 can decrease *PAICS* expression by directly interfering with MYC binding to its promoter (**Fig. 4B**).Moreover, Barfeld et al., demonstrated

that the purine biosynthetic pathway genes-PAICS and IMPDH2 (IMP (Inosine 5'-Monophosphate) Dehydrogenase 2) are regulated by MYC in prostate cancer (8).

DISCUSSION

Next-generation sequencing data have identified molecular and genetic features that reveal prostate cancer as a clinically heterogeneous disease (31). Nearly 90% of prostate-specific antigen (PSA) screening identifies PCa to be localized at diagnosis (32). Anti-androgen therapies that target the androgen receptor remain important early and therapeutic strategy that has improved survival. However, a sizeable patient population develops resistant variants that are aggressive and metastatic. Clinical heterogeneity in this population leads to poor prognosis and therefore dearth of therapeutic options. Biomarkers that have clear diagnostic and predictive value will lead to risk stratification and potential systemic therapies. Recently, we demonstrated that *PAICS* expression correlated with lung cancer progression (22). In this study, we have identified *PAICS* expression quantitatively correlate with different stages of prostate cancer where it is negligible in benign PCa, moderately expressed in localized PCa and intensely expressed in metastatic PCa. Further, we see concurrence of *PAICS* expression with respect to transcript, protein and immunohistochemistry in patient samples. PAICS catalyzes the production of Phosphoribosylaminoimidazolesuccinocarboxamide (SAICAR), an intermediate metabolite in d*e novo* purine biosynthetic pathway (33,34).

Through several *in vitro* and *in vivo* studies we demonstrate that PAICS is necessary for prostate cancer proliferation and invasion. Specifically, we show that PAICS function is imperative in DU145 and PC3 both of which are androgen-independent prostate cells. Thus we extrapolate that PAICS function is necessary and therefore a possible therapeutic target in androgen independent

castration resistant prostate cancer (CRPC) clinical settings. Furthermore, since *de novo* purine biosynthetic pathway genes including PAICS is expressed in transformed cells as compared to salvage pathway in normal cells; therapeutic targeting of PAICS can avoid toxicity issues. Moreover, SAICAR has potential value as non-invasive diagnostic marker and chemical mimetic with therapeutic implications.

Recent molecular analyses of 333 primary prostate carcinomas identified ~26% subset (of good and poor clinical prognosis) with unexplained molecular alterations that identified amongst others, amplifications of chromosome 8 spanning myc. *MYCN* was identified to be amplified in prostate cancer with lymph node metastasis (2). *MYCN* was also identified as oncogene in CRPC-neuroendocrine cancer (2). Additionally, *MYC* amplification and over-expression predicts poor outcome. MYC also has well-demonstrated role in several anabolic pathways such as amino acid metabolism and purine biosynthesis. Previous work showed that inducible MYC over-expression increased expression of *de novo* purine biosynthesis pathway enzymes including PAICS, in LnCaP (an androgen responsive PCa cell line) (7,8). Though role of MYC as oncogene remains indisputable, its role as transcription factor has made it difficult to be therapeutically targeted. The “undruggability” of MYC has been circumvented by synthetic lethal approaches (10) or targeting genes that are either directly or indirectly regulated by MYC. In this study, we identified JQ1, the bromodomain inhibitor concurrently alleviates both MYC and PAICS expression across several PCa cells that are both androgen sensitive and independent. We demonstrate that JQ1 mediates abrogation of PAICS expression by directly reducing MYC occupancy on *PAICS* promoter. This study furthers previous observation of MYC mediated *de novo* purine biosynthetic pathway regulation by identifying JQ1 as potential therapeutic reagent for MYC regulated gene, PAICS in prostate cancer cells.

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Figure legends

Figure 1. *De novo* purine biosynthetic enzyme PAICS shows increased expression in prostate cancer. (A) Gene expression profiling analysis of multiple prostate cancer datasets using Oncomine database suggest *PAICS* expression across the datasets (35-44) between normal prostate and primary prostate cancer. (The rank for a gene is the median rank for that gene across each of the analyses. The p-value for a gene is its p-value for the median-ranked analysis.). **(B)** Transcriptome sequencing of prostate cancer. *PAICS* expression in benign, primary and metastatic prostate cancer were measured in RPKM (read per million Kilobase). (**C)** Expression of PAICS in normal prostate, primary and metastatic tumor samples from TCGA. **(D)** Immunoblot analysis of *PAICS* using lysates from benign prostate, primary prostate and metastatic prostate cancer tissue lysates. β-actin served as a loading control. **(E)** Immunohistochemical staining of prostate tissue with *PAICS* specific antibody. **(F)** The staining was scored and the intensity was measured as product score and plotted as box plots.

Figure 2. Purine biosynthetic pathway enzyme PAICS is required for prostate cancer cell proliferation and invasion. (A) Immunoblot analysis of PAICS using lysates from aggressive prostate cancer cell lines DU145 and PC3 treated with two specific and independent *PAICS* siRNA duplexes or non-targeting siRNA. β-actin is used as a loading control. (**B)** Knockdown of PAICS reduced the prostate cancer cell proliferation and (**C**) colony formation and (**D**) invasion in Boyden chamber matrigel invasion assay, respectively.

Figure 3. PAICS plays a role in prostate cancer growth and metastasis *in vivo*. (A) Immunoblot analysis of PAICS knockdown cell lysates using two independent shRNAs in DU145 cells. (**B)** The PAICS knockdown cells were utilized in the *in vivo* chicken chorioallantoic membrane (CAM) assay. Tumor growth was measured in the knockdown as well as in control DU145 non-targeting shRNA cells. Tumor size plotted corresponds to average

tumor size of 8 eggs per group. (**C)** and **(D)** PAICS knockdown reduces intravasation and metastasis of DU145 cells in the CAM models. Metastasized cells to the lower CAM and liver of chicken embryos were quantified using human Alu specific PCR. (**E)** PAICS knockdown in PC3 cells inhibits tumor growth in a mouse xenograft model. Athymic nude mice were injected with PC3 cells that had either stable PAICS knockdown or non-targeting shRNA and tumors were monitored at indicated time points, and plotted. Inset: Immunoblot analysis of PAICS using these stable knockdown lysates. (**F)** Tumor weights of corresponding mouse xenograft models. Non-targeting shRNA was used as a control. The solid black line is for Non-T shRNA, the dashed line is for shRNA1 and dotted line is for shRNA2. N=8 mice per group; **P*<0.006, compared with non-targeting shRNA xenografts.

Figure 4. Bromodomain inhibitor JQ1 inhibits PAICS expression. Prostate cancer cells were treated with JQ1 for 48 h. **(A)** Decreased MYC and PAICS expression in prostate cancer cells treated with JQ1 (1 and 5 uM) by immunoblot analysis. β-actin is used as a loading control. (**B)** and **(C)** Chromatin immunoprecipitation analysis for the MYC occupancy on *PAICS* promoter in androgen sensitive prostate cancer cell lines LnCaP and VCaP following JQ1 or DMSO treatment for 48 h. (**B)** Schematic representation of the *PAICS* genomic region showing gene and amplicon positions. ChIP-qRTPCR analysis using various primers designed at indicated positions. ChIP was performed using antibodies against MYC and a control IgG. Error bars: n = 3. All bar graphs are shown with ± SEM.

Figure 1 .

Figure 2 .

Figure 3 .

Figure 4 .

Suppl Figure 1 .

Suppl Figure 2 .

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