Reprogramming of Isocitrate Dehydrogenases Expression and Activity by the Androgen Receptor in Prostate Cancer

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Abstract

Mutations of the isocitrate dehydrogenase genes IDH1 and IDH2, key enzymes involved in citrate metabolism, are important oncogenic events in several cancer types, including in 1%–3% of all prostate cancer cases. However, if IDH1 and other IDH isoforms are associated with prostate cancer progression, as well as the regulatory factors controlling their expression and activity, remain mostly unknown. Using publicly available datasets, we showed that prostate cancer harbors the highest IDH1 expression across the human cancer spectrum and that IDH1 expression is altered during prostate cancer progression. We showed that the androgen receptor (AR), a key oncogene in prostate cancer, controls multiple IDH isoforms in both in vitro and in vivo models, predominantly positively regulating IDH1. Chromatin immunoprecipitation experiments confirmed the recruitment of AR at several regulatory regions of IDH1 and enzymatic assays demonstrated that AR significantly induces IDH activity. Genetic blockade of IDH1 significantly impaired prostate cancer cell proliferation, consistent with IDH1 having a key function in these cancer cells. Importantly, knockdown of IDH1 blocked the AR-mediated induction in IDH activity, indicating that AR promotes a mitochondrial to cytoplasmic reprogramming of IDH activity. Overall, our study demonstrates that IDH1 expression is associated with prostate cancer progression, that AR signaling integrates one of the first transcriptional mechanisms shown to regulate IDH1, and that AR reprograms prostate cancer cell metabolism by selectively inducing extra-mitochondrial IDH activity.

Implications: The discovery that AR reprograms IDH activity highlights a novel metabolic reprogramming necessary for prostate cancer growth and suggests targeting IDH activity as a new therapeutic approach for prostate cancer treatment.

Introduction

Prostate cancer is an androgen-dependent disease that relies on the androgen receptor (AR) for cancer cell proliferation and survival, and thus inhibiting AR activity is currently the cornerstone therapy in clinical settings (1, 2). Despite the positive response of most cancer cases to AR blockage, evolution of the disease to castration-resistant prostate cancer (CRPC) is almost inevitable (1–4), highlighting the need to develop novel therapies for prostate cancer. In >80% of all cases, resistance involves AR reactivation (5–8). Cancer cells can also evolve into AR-negative (AR−) prostate cancer, which is often referred to as neuroendocrine prostate cancer (NEPC; ref. 9).

Glucose, amino acids, and fatty acids can fuel mitochondrial activity through the tricarboxylic acid (TCA) cycle for ATP production and to sustain biosynthesis of essential cellular components (e.g., nucleotides and lipids). The prostate is metabolically unique in human in that epithelial prostate cells have a blunted TCA cycle to produce high levels of citrate (10, 11). One of the earliest events in prostate cancer, which occurs in virtually all cancer cases, is the reprogramming of this unique metabolic program (10, 11). Given its major oncogenic functions in prostate cancer, it is not surprising that AR was identified as a major orchestrator of energy metabolism and that prostate cancer progression was linked to specific metabolic gene signatures (12–18). Moreover, AR was shown to alter citrate metabolism, most notably by inducing mitochondrial respiration and dysregulating lipid metabolism, contributing to the rising characterization of AR functions in the transcriptional control of energy metabolism (12, 14, 15). However, what are the key limiting enzymatic steps under the AR-driven metabolic program remain unknown.

It is now well recognized that mutations of isocitrate dehydrogenase genes IDH1 and IDH2 lead to the establishment of an oncometabolic environment that causes cancer, notably by altering the epigenome (19, 20). These mutations, which induce a novel enzymatic activity catalyzing the formation of 2-hydroxyglutarate, interfere with α-ketoglutarate (αKG)-dependent epigenetic regulatory mechanisms and lead to the

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hypermethylation of the genome (20–23). Although frequent in gliomas and other cancer types, IDH1 mutations occur in only 1%–3% of prostate cancer and are believed to be the underlying oncogenic drivers in these cases (24–28). On the basis that the prostate harbors a distinct citrate metabolic program, we hypothesized that an alteration in the regulation of IDH enzymes could be linked to prostate cancer development and progression, even in prostate cancer cases without IDH1 mutation.

IDHs are encoded by five different genes leading to three possible functional IDH enzymatic complexes (IDH1, IDH2, and IDH3, the latter comprising IDH3A, IDH3B, and IDH3G). In the mitochondria, either the IDH2 or the IDH3 complexes catalyze the decarboxylation of isocitrate, produced from citrate, into αKG using, respectively, NADP⁺ and NAD⁺ as cosubstrates. This represents a key step in the mitochondrial TCA cycle. In addition, cytoplasmic IDH1 promotes the NADP⁺-dependent production of extra-mitochondrial αKG, which is required for the proper activity of several enzymes involved in epigenetic regulation (20–23). Newly synthesized NADPH can be used for anabolic processes such as lipogenesis. However, what are the molecular mechanisms controlling IDH gene expression and how these dynamics are altered in prostate cancer cell metabolism are still unknown.

In this study, we observed that wild-type IDH1 is predominantly expressed in prostate cancer cells and that its expression is altered throughout prostate cancer progression. We identified AR as a key regulator of several IDH isoforms, most notably IDH1. We showed that, for no more than 3 months after resuscitation and androgen stimulation, IDH1 activity, favoring cytoplasmic over mitochondrial metabolism, importantly, knockdown of IDH1 decreased cancer cell proliferation and a blockade of the IDH metabolic reprogramming, possibly highlighting IDH1 as a potential new therapeutic target for prostate cancer.

Materials and Methods
Cell culture
LNCaP, LAPC4, 22rv1, PC3, VCaP, and DU145 cells were grown in RPMI supplemented with 10% FBS, penicillin, streptomycin, and 1% L-glutamine. For androgen stimulation, cells were harvested in PBS and counted using an automated cell counter TC10 (Bio-Rad). For basal activity, cells were harvested after 48 hours in complete media. For androgen stimulation, steroid deprivation was performed for 48 hours in media with 5% CSS before 48-hour stimulation with R1881 in media with 5% CSS. After cell count, cells were resuspended in ice-cold IDH assay buffer at a 1 million cells per 200-μL concentration. Cells were then divided 1:8 in IDH assay buffer before the enzymatic activity assay. For purification of cytoplasmic and mitochondrial extracts for IDH enzymatic activity, cellular fractionation was performed according to the protocol of Gravel and colleagues (29).

RNA extraction and quantitative reverse transcription PCR
Isolation and purification of total RNA were performed using the EZ-10 RNA extraction kit from Bio-Rad. The Luna Universal qPCR Master Mix (NEB) was then used for qPCR and quantification of specific genes, and duplicate technical replicates were performed for every biological sample. Relative expression was normalized to the expression of two housekeeping genes, PUM1 and TBP. Primer sequences are detailed in Supplementary Table S1. Results are shown as the average of 2–4 independent experiments with at least 3 biological replicates per condition and the Student t test was used to evaluate statistical significance. For RNA-seq analysis, raw reads were aligned to the human genome (GRCh38) using the HISAT2 alignment tool (30) and trimmed using Trimmomatic (31). Alignment and quantification of the samples to the transcriptome hg38 were then performed using Kallisto (32). RNA-seq data are available on the Gene Expression Omnibus (GEO: GSE128201).

ChIP-qPCR
Chromatin immunoprecipitation (ChIP)-qPCR was performed in LNCaP and 22rv1 cells using an anti-AR antibody (sc-7305, Santa Cruz Biotechnology) as described previously (13). In brief, after treatment with R1881 for 16 hours, cells were cross-linked in 1% formaldehyde for 10 minutes at room temperature. Nuclei were then purified using sequential centrifugation and chromatin was sonicated. Immunoprecipitation was then performed overnight at 4°C, and AR-bound chromatin was enriched using
Dynabeads (Life Technologies). DNA was then purified using the fast ChIP protocol (33). For ChIP-qPCR, cells treated with vehicle were used as controls of antibody nonspecific binding, and two to three negative regions were used for ChIP normalization between samples. Sequences of primers used for AR DNA binding–positive and negative regions are detailed in Supplementary Table S2. Results are shown as the average of three independent experiments. ChIP-seq experiments were performed using the same protocol with the AR antibody sc-816X from Santa Cruz Biotechnology (Audet-Walsh and colleagues, in preparation) and analyzed as described previously (15). Visualization was performed using the UCSC genome browser following the merging of two independent experiments.

**Clinical data and statistical analyses**

IDH gene expression data across the human cancer spectrum and in prostate cancer specimens were obtained from The Cancer Genome Atlas (TCGA) consortium (34, 35) and from the Human Protein Atlas website in July 2018 (www.proteinatlas.org; ref. 36). Data from the Cancer Cell Line Encyclopedia (CCLE), including shRNA results from the Achilles project, were obtained from the webportal (https://portals.broadinstitute.org/ccle) in November 2018 (37). For the Kaplan–Meier and the log-rank test, data from Taylor and colleagues (GSE21032) were analyzed through the Project Betastasis webpage (www.betastasis.com; ref. 8). Expression data from six benign prostate tissues, seven clinically localized primary prostate tumors, and six hormone-refractory metastatic prostate tumors were described by Varambally and colleagues (GSE3325; ref. 38). Expression data from 28 benign prostate tissues, 59 localized prostate cancer, and 35 metastatic CRPC were described by Grasso and colleagues (GSE35988; ref. 39). The microarray data from Sun and colleagues (40) were analyzed for IDH gene expression in human LuCaP35 prostate tumor explants (5 mice with sham surgery and 5 castrated; GDS4120). RNA-seq from xenograft experiments with 22rv1 cells and their metastatic subclones were obtained from the Tsai and colleagues' dataset (GSE99857; ref. 41).

**Results**

**IDH1 is highly expressed in prostate cancer and is altered during prostate cancer progression.**

To better understand the relationship between IDH gene expression and prostate cancer, we screened for their mRNA levels across the human cancer spectrum using The Cancer Genome Atlas dataset (TCGA; refs. 34, 35). All five IDH genes showed ubiquitous expression across the diverse types of human cancer interrogated (Fig. 1; Supplementary Fig. S1), but only IDH1 had a much higher median in prostate cancer compared with other cancer types analyzed (Fig. 1A). The median IDH1 expression level in prostate cancer samples was even about 3-fold higher than in gliomas, a tumor type in which IDH1 mutation is known as a key oncogenic driver linked to disease development (42). Similarly, prostate cancer cell models had the second highest IDH1 expression levels in the Cancer Cell Line Encyclopedia (Fig. 1B). On the basis of these results, we hypothesized that IDH1 could be an important metabolic enzyme associated with prostate cancer cell metabolism.

In human prostate cancer samples, we found that low expression levels of IDH1 were associated with a high recurrence rate following radical prostatectomy ($P = 0.0473$; Fig. 2A). Although prostate cancer has high basal IDH1 expression levels, lower levels are associated with more aggressive diseases. Consistent with this observation, IDH1 levels were significantly lower in metastatic CRPC in comparison with those of both primary tumors and benign normal prostate samples from the Grasso and colleagues' (39) dataset (Fig. 2B). These results were validated in a second cohort with similar results (Fig. 2C). Indeed, the four microarray probes targeting IDH1 indicated significantly lower levels in metastatic prostate cancer compared with primary tumors and/or benign normal prostate. These results indicate that IDH1 expression is altered during prostate cancer progression and that more aggressive diseases have lower IDH1 levels.

Given the major role of AR in prostate cancer, we were intrigued about the possible relationship between IDH1 expression, prostate cancer progression, and AR status. We hypothesized that evolution of AR$^+$ to AR$^−$ (neuroendocrine) prostate cancer tumors favors a metabolic reprogramming involving the loss of IDH1. Using the Grasso and colleagues' dataset (39), we separated CRPC samples in two groups. We first established whether AR-driven CRPC tumors had increased AR expression compared with primary tumors ($n = 29$). Second, we regrouped CRPC tumors that had decreased AR expression compared with primary tumors concomitantly with the increased expression of the neuroendocrine markers ENO2 and CHGA ($n = 6$). As expected, we observed a significant difference in AR expression levels compared with ENO2 and CHGA expression in these two subsets of CRPC, categorized respectively as AR$^+$ and neuroendocrine CRPC (Fig. 2D). Consistent with our hypothesis on IDH1 reprogramming following loss of AR, IDH1 levels were significantly lower in NEPC compared with tumors driven by ARs (Fig. 2D). To further link IDH1 expression with AR expression and activity, we performed correlation analyses of IDH1 expression in relationship with mRNA levels of AR, ENO2, and CHGA using the TCGA cbioPortal to access the TCGA, the Taylor and colleagues (8), and the Kumar and colleagues clinical datasets (43). Interestingly, IDH1 levels were significantly correlated with AR expression ($P > 0.00005$) in the TCGA dataset, with small yet significant inverse correlation with both NEPC markers ($P < 0.05$; Fig. 2E). We next studied these correlations in a clinically more aggressive setting with the Taylor and the Kumar and colleagues' datasets. First, in the Taylor and colleagues' dataset (Fig. 2F), IDH1 was also found to be significantly and positively correlated to AR but inversely correlated to ENO2 and CHGA ($P < 0.0007$). IDH1 was also positively correlated with AR ($P < 3 \times 10^{-5}$) and inversely correlated with ENO2 and CHGA ($P < 2 \times 10^{-5}$) in the Kumar and colleagues' dataset (Fig. 2G). Altogether, these results demonstrate that IDH1 expression is altered during prostate cancer progression and that it is correlated with AR expression.

**AR controls IDH1 expression in prostate cancer cells.**

We then studied the relative expression of the five IDH genes in in vitro models of AR-positive (AR$^+$) human prostate cancer (LNCaP, LAPC4, and 22rv1 cells; Fig. 3A). In AR$^+$ prostate cancer cells, IDH1, which encodes the only cytoplasmic IDH enzyme, was the most expressed gene in prostate cancer cells compared with the mitochondrial isoforms (Fig. 3A). IDH2, IDH3A, IDH3B, and IDH3G had similar expression levels, which ranged between 7- and 321-fold lower than IDH1 expression in LNCaP, LAPC4, and 22rv1 cells (Fig. 3A). The only different profile was observed in VCaP cells, which exhibited similar levels of IDH1 and IDH3.
genes. We also performed RNA-seq analyses in LNCaP and 22rv1 cells. After normalization, these data also demonstrate that IDH1 is the predominant IDH isoform expressed in these ARþ prostate cancer cells (Fig. 3B and C). We validated our results by reanalyzing publicly available RNA-seq experiments in 22rv1 cells, which also indicated that IDH1 is the most highly expressed IDH isoform in these cancer cells in vivo (Fig. 3D). Tsai and colleagues used 22rv1 cells in vivo to develop a highly metastatic prostate cancer model (41). Reanalyses of their data indicate that, as seen in vitro for 22rv1 cells (Fig. 3C and D), IDH1 was also the most highly expressed IDH isoform in vivo, with expression levels ranging from 4- to 13-fold higher than levels of other IDH genes (Fig. 3E, light green). Interestingly, the metastatic subline also showed a predominance of IDH1 expression, but that was statistically decreased compared with the parental cell lines (Fig. 3E, dark green). We then studied IDH gene expression levels in AR/C0 human prostate cancer cells (PC3 and DU145). These cancer cells harbored a different IDH expression pattern compared with ARþ prostate cancer cells (Fig. 3A), as IDH1 levels were similar to those of IDH3s (Fig. 3F). We also performed RNA-seq analyses in PC3 cells. Contrary to ARþ cells, IDH1 was not expressed at a higher level compared with other IDH isoforms (Fig. 3G). Accordingly, total IDH activity was significantly higher in ARþ prostate cancer cells, with 2- to 10-fold higher activity than in ARþ prostate cancer cells (Fig. 3H). Overall, these results indicate that IDH1 is the predominant IDH gene expressed in ARþ prostate cancer cells in vitro and in vivo.

We next investigated the relationship between AR activation and the regulation of IDH expression. Treatment of LNCaP cells with the synthetic androgen R1881 significantly induced the expression of the prostate-specific antigen (PSA) mRNA (encoded by KLK3; Fig. 4A, left). AR activation also significantly increased the expression of IDH1 (Fig. 4A, right). IDH2 and members of the IDH3 complex were much less stimulated, if at all, by R1881 (Fig. 4A, right). As in LNCaP cells, IDH1 was signifi-
cantly induced in VCaP (Fig. 4B) and 22rv1 (Fig. 4C) cells following R1881 treatment. No other IDH genes were significantly modulated by AR in these cells, except a trend for an increase in IDH3A in VCaP cells. In LAPC4 cells, IDH2, IDH3A, and IDH3G were moderately induced by androgens (Supplementary Fig. S2). It should be noted that LAPC4 cells harbor the highest basal IDH1 expression levels across all cell lines tested (Fig. 3A). In human prostate cancer xenografts of LuCaP35, inhibition of the androgen signaling pathway by castration led to a significant decrease in several IDH genes in vivo (Fig. 4D). Indeed, several microarray probes targeting IDH1, IDH2, and IDH3A showed a significant decrease after castration, sustaining a positive regulation by androgens of these genes in prostate cancer cells in vivo.

AR ChIP-seq analyses identified two and three AR DNA–binding sites in the vicinity of IDH1 and IDH2, respectively, in LNCaP cells (Fig. 4E; Supplementary Fig. S3A). In the absence of...
androgens, no peak was observed, while R1881 treatment significantly induced AR recruitment to \textit{IDH1} (Fig. 4E). No recruitment of AR was detected for \textit{IDH3A}, \textit{IDH3B}, or \textit{IDH3G} in ChIP-seq analyses from LNCaP cells (Supplementary Fig. S3B–S3D). Similarly, reanalysis of AR ChIP-seq experiments from Massie and colleagues (16) and Singh and colleagues (44) also indicates a significant AR DNA–binding site 2 kb upstream \textit{IDH1} transcriptional start site (corresponding to \textit{IDH1} #1, Fig. 4E). Validation was performed using ChIP-qPCR experiments, which confirmed the recruitment of AR to genomic DNA regions in proximity to the \textit{IDH1} promoter in LNCaP cells (Fig. 4E). Given that AR binding to these sites was relatively low, particularly for the second site, we cannot exclude that AR also controls \textit{IDH1} expression in an indirect manner. Complementary validation was performed in 22rv1 cells (Fig. 4E). In accordance with the qPCR results showing that only \textit{IDH1} is increased by R1881 in 22rv1 cells (Fig. 4C), AR was exclusively recruited to \textit{IDH1} promoter in ChIP-qPCR analysis (Fig. 4E), while no recruitment was observed at \textit{IDH2} (Supplementary Fig. S3A). These results indicate that AR controls the expression of \textit{IDH1}, thus identifying one of the first transcription factors controlling this gene expression in the context of prostate cancer.

**IDH1 modulation reprograms prostate cancer cell metabolism**

To understand the functional role of \textit{IDH1} in AR\textsuperscript{+} prostate cancer cells, we performed \textit{IDH} enzymatic activity assays. After AR activation in LNCaP cells, a significant induction of 3.8-fold of total \textit{IDH} activity was observed (Fig. 5A). Similarly, R1881 treatment led to a 5.3-fold induction of total \textit{IDH} activity in VCaP cells (Fig. 5B), correlating with a positive regulation of \textit{IDH1} expression by AR in these cancer cells (Fig. 4). To further delineate the contribution of \textit{IDH1} (in the cytoplasm and peroxisomes) and \textit{IDH2/3} (in the mitochondria) to the total \textit{IDH} activity in prostate cancer cells, with and without AR
activation, we performed siRNA-mediated knockdown of IDH1 in LNCaP cells. As expected, transfection of a pool of siRNAs targeted against IDH1 significantly decreased its expression by >90% (Fig. 5C). Importantly, this knockdown was selective and did not affect other IDH gene expression (Fig. 5C). IDH1 knockdown, in absence of androgens, led to a 3.7-fold decrease in total IDH activity in LNCaP cells, indicating that IDH1 represents the major active isoform in these cells (Fig. 5D), correlating with IDH expression data (Fig. 3A). Importantly, IDH1 knockdown completely blocked the AR-mediated induction in total IDH activity, with the loss of 87% of total IDH activity in LNCaP cells (Fig. 5D). Similarly, we observed in VCaP cells that IDH1
knockdown was specific and did not affect other IDH gene expression (Fig. 5E). As anticipated, IDH1 knockdown completely blocked the R1881-mediated increase in IDH activity in VCaP cells (Fig. 5F).

Loss of IDH1 in LNCaP cells significantly decreased proliferation in complete media (Fig. 6A). Similarly, loss of IDH1 also decreased proliferation in absence of androgens, indicating that IDH1 is required for maximal proliferation (Fig. 6B). The reanalysis of the Achilles project data, which uses genome-scale RNAi and CRISPR-Cas9 to silence or knockout gene expression across hundreds of cancer cell lines (45, 46), also confirmed that prostate cancer cells are one of the most sensitive cancer types to the loss of IDH1 (Supplementary Fig. S4). This seemed to be mostly specific to AR+ cells as IDH1 knockdown in PC3 and DU145 AR- cells did not significantly or only modestly change proliferation (Supplementary Fig. S5). These data suggest that an induction of cytoplasmic/mitochondrial IDH activity is required for maximal prostate cancer cell proliferation driven by AR. We thus performed cytoplasmic and mitochondrial preparations known to preserve metabolic activities (29). In extracts from R1881-treated cells, we observed a significant 3.24-fold induction of the cytoplasmic IDH activity, further indicating that IDH1 is the major target of AR in LNCaP (Fig. 6C) cells. Importantly, no increase in

**Figure 4.**
IDH gene expression regulation by AR in prostate cancer cells. A, Relative mRNA expression of IDH genes in LNCaP cells after 24 hours of treatment with 10 nmol/L R1881 (right). KLK3 expression, which encodes the prostate-specific antigen (PSA), is shown as a positive control (left). B, Relative mRNA expression of IDH genes in VCaP cells after 24 hours of treatment with 10 nmol/L R1881 (right). KLK3 expression is shown as a positive control (left). IDH2 was barely detectable and is not shown. C, Relative mRNA expression of IDH genes in 22rv1 cells after 24 hours of treatment with 10 nmol/L R1881 (right). FASN expression is shown as a positive control (left). For A, B, and C, results are shown as the mean ± SEM of 2 to 4 independent experiments performed with triplicate biological replicates. D, IDH gene expression following castration in human prostate cancer xenografts of LuCaP35 (n = 5/group). Log-transformed data are shown normalized to controls (sham surgery). E, Analysis of AR recruitment to IDH1 after treatment with R1881. The UCSC genome browser view is shown for each gene as well as AR ChIP-seq signal intensity for control and R1881-treated LNCaP cells. The AR fold enrichment in LNCaP and 22rv1 cells in ChIP-qPCR validation experiments are indicated below the genome browser views (the averages of three independent experiments are shown). Insets indicate ChIP-qPCR validation of AR recruitment at the -12.5 kb enhancer of the KLK3 gene. *P < 0.05; **P < 0.01; ***P < 0.001, Student t test.
mitochondrial IDH activity was observed in extracts from R1881-treated cells (Fig. 6C). Knockdown of IDH1 completely blocked this induction of cytoplasmic IDH activity by AR in LNCaP cells (Fig. 6D), demonstrating that AR promotes a reprogramming of IDH activity favoring cytoplasmic over mitochondrial metabolism.

**Discussion**

In this study, IDH1 was shown to be the predominant IDH gene expressed in prostate cancer and to be tightly linked with AR expression and activity. Accordingly, IDH1 was shown to be sensitive to AR activation in both in vitro and in vivo prostate cancer models. We further identified AR DNA–binding sites close to the promoters of IDH1 that were validated by ChIP-qPCR. Using siRNAs, we demonstrated that loss of IDH1 significantly impairs proliferation, but also blocks the reprogramming by AR of cytoplasmic to mitochondrial IDH activity. Consistent with a reprogramming of cell metabolism during carcinogenesis, IDH1 gene expression was significantly altered during prostate cancer progression. Overall, our study highlights the importance of the wild-type IDH1 gene in prostate cancer.

In gliomas, glioblastomas, and certain types of leukemia, mutations in IDH1 are frequently identified as a major oncogenic driver (19, 20). IDH1 mutations can occur in prostate cancer but are generally rare (24–28). Given the predominance of IDH1 mutations in other cancer types, it was surprising to observe such a high expression in prostate cancer samples. Rather than an overexpression in prostate cancer primary tumors, IDH1 expression is not different compared with peritumor normal tissues (Fig. 2), suggesting that IDH1 levels are normally high in the prostate. This might be related to the citrate-secretory phenotype of prostate epithelial cells that require a unique metabolic program to sustain citrate production but also to control intracellular citrate levels and usage. Indeed, prostate epithelial cells exhibit a truncated mitochondrial citric acid cycle to allow the massive production of citrate, which is subsequently secreted (11).
This citrate-secretory phenotype is lost early in the course of prostate cancer, at a stage where the tumor is still localized. The decrease of IDH1, observed in metastatic CRPC samples, probably reflects a subsequent change in citrate metabolism not directly linked to the loss of the prostate citrate-secretory phenotype that occurs earlier. It is not completely clear why IDH1 is decreased in metastatic CRPC samples compared with primary tumors. One explanation is that these tumors comprise 15%–20% of NEPC samples, induced upon sequential androgen treatments, which are characterized by low AR and IDH1 expression (Fig. 2). This is probably why low IDH1 expression is linked to faster recurrence (Fig. 2A), possibly indicating more aggressive prostate cancer. In addition, it has been shown that cancer cells further undergo metabolic reprogramming during establishment at distal metastatic sites, notably in the breast cancer setting (47). This phenomenon most probably also occurs during prostate cancer metastasis establishment, which is supported by the 22rv1 metastatic model (Fig. 3E). Nevertheless, IDH1 is still highly expressed in AR+ prostate cancer cells derived from patients with CRPC, such as the cellular models used in this study, as well as responsive to androgens.

To better understand the role of IDH1 in prostate cancer, we performed our studies in AR+ prostate cancer cells, which better represent clinically localized and locally advanced prostate cancer as they are characterized by a dependency on AR signaling. Accordingly, in VCaP, LNCaP, and 22rv1 cells, IDH1 was significantly induced following treatment with R1881 while other IDHs were not consistently modulated upon AR activation. This regulation by androgens was observed in several prostate cancer models and correlation studies in human prostate cancer samples also support the concept that AR controls IDH1 expression. Mostly studied for its enzymatic activity, not much is known about IDH1 gene regulation, and thus AR appears as one of the first transcriptional regulatory mechanisms controlling its expression. Indeed, AR activation induced its expression by up to 4-fold and, more importantly, also induced its activity up to 6-fold in prostate cancer cells. It is also possible that AR controls IDH1 expression and activity in other contexts, such as in cancers with IDH1 mutation, and it is tempting to speculate that we could use androgens in these cases to decrease the expression of mutant IDH1 oncogenic enzymes.

By specifically inducing IDH1 activity, AR promotes an unexpected metabolic rewiring in prostate cancer cells, favoring cytoplasmic/mitochondrial IDH ratios, which was associated with prostate cancer cell proliferation status. Given that IDH1 is an important pathway for the replenishment of NADPH, its increase could favor biosynthetic pathways that rely on this cofactor for proper activity, such as lipid synthesis that is also activated by AR in prostate cancer cells (12, 15). In addition, IDH1 promotes the synthesis of oxK, which is an important cofactor for several proteins associated with the epigenome modulation. How modulation of oxK levels after modulation of IDH1 will affect the epigenome regulation has yet to be determined and mechanistic studies are required to fully capture the implication of IDH1 activity in prostate cancer cell genomic activity.

In prostate cancer samples, IDH1 expression is correlated to AR expression and decreased during the transition of prostate cancer to AR–NEPC. IDH1 expression was significantly lower in CRPC human samples compared with primary tumors in two independent datasets. Interestingly, a similar pattern was observed in 22rv1 xenograft experiments, in which the highly metastatic subclone also had significantly lower levels of IDH1 compared with the parental cell line. The loss of IDH1 expression in AR+ prostate cancer cells could be a consequence of the absence of a functional AR pathway in these cells. Given that IDH1 expression is required for maximal proliferation in AR+ prostate cancer cells and that it is associated with a significant metabolic reprogramming, these results suggest that loss of IDH1 is an important step for the evolution to metastatic or NEPC cancer cells. Further studies are required to fully understand the implication of IDH1 in the metastatic potential of prostate cancer cells.
In summary, wild-type IDH1 is an important metabolic gene expressed in the prostate and for which expression is altered during prostate cancer progression. Importantly, the androgen signaling pathway was identified as one of the first transcriptional regulatory mechanisms that govern IDH1 expression and activity. Indeed, AR was shown to positively regulate IDH1 expression in several prostate cancer models, analogous with a positive correlation between AR and IDH1 expression in human prostate cancer samples. Consequently, AR activation led to a reprogramming of total IDH activity, favoring a cytoplasmic/mitochondrial IDH ratio to sustain prostate cancer cell proliferation. Overall, our study highlights the key role played by IDH1 in AR+ prostate cancer cell metabolism and suggests it could be used as a novel therapeutic target for the treatment of this disease before evolution to NEPC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: K. Gonthier, E. Audet-Walsh
Development of methodology: K. Gonthier, C. Weidmann, E. Audet-Walsh
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