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The Long Non-Coding RNA *PCAT-1* Promotes Prostate Cancer Cell Proliferation through cMyc[1,2]

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Abstract

Long non-coding RNAs (lncRNAs) represent an emerging layer of cancer biology, contributing to tumor proliferation, invasion, and metastasis. Here, we describe a role for the oncogenic lncRNA *PCAT-1* in prostate cancer proliferation through cMyc. We find that *PCAT-1*–mediated proliferation is dependent on cMyc protein stabilization, and using expression profiling, we observed that cMyc is required for a subset of *PCAT-1*–induced expression changes. The *PCAT-1*–cMyc relationship is mediated through the post-transcriptional activity of the *MYC* 3′ untranslated region, and we characterize a role for *PCAT-1* in the disruption of *MYC*-targeting microRNAs. To further elucidate a role for post-transcriptional regulation, we demonstrate that targeting *PCAT-1* with miR-3667-

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3p, which does not target *MYC*, is able to reverse the stabilization of cMyc by *PCAT-1*. This work establishes a basis for the oncogenic role of *PCAT-1* in cancer cell proliferation and is the first study to implicate lncRNAs in the regulation of cMyc in prostate cancer.

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Introduction

The maturation and spread of high-throughput sequencing technologies has enabled increasingly complex analyses of the cellular transcriptome, with the nomination of numerous novel RNA species [1,2]. Among these, long non-coding RNAs (lncRNAs) over 200 bp in length have been implicated as fundamental actors in numerous molecular processes, including cell differentiation, lineage specificity, neurologic disorders, and cancer [3–5].

In prostate cancer, the first prominent lncRNA, *PCA3*, was initially described as a novel biomarker of disease [6] and subsequently defined as a promising urine test for this disease [7]. Similarly, the lncRNA *PCGEM1* has been implicated in prostate cancer as a regulator of apoptosis [8] and newer lncRNAs are being pursued through high-throughput technologies [9]. Using next-generation sequencing of a large cohort of prostate cancer tissues, we recently defined the landscape of lncRNAs in prostate cancer, including *SChLAP1* as a regulator of metastasis [10,11]. On the basis of this unbiased whole-transcriptome analysis, we have previously nominated *PCAT-1* as the top-ranked, upregulated lncRNA in prostate cancer tissues [10], and independent studies have confirmed overexpression of *PCAT-1* in prostate cancer [9] and implicated *PCAT-1* as a prognostic biomarker for colorectal cancer metastasis and poor patient survival [12].

Here, we characterize the function of the lncRNA *PCAT-1* in prostate cancer. We show that *PCAT-1* promotes prostate cell proliferation and that this phenotype is mediated through up-regulation of the cMyc protein (encoded by the *MYC* gene). Antagonism of cMyc is able to reverse *PCAT-1*–mediated cell proliferation. We show that *PCAT-1* regulates cMyc post-transcriptionally through the *MYC* 3′ untranslated region (UTR). Further, we find a protective effect of *PCAT-1* on cMyc by interfering with the regulation of *MYC* by miR-34a. Conversely, microRNA-based targeting of *PCAT-1* reverses its effect on cMyc. These studies elucidate a novel mechanism of cMyc stabilization in prostate cancer and describe a post-transcriptional function of *PCAT-1* in the antagonism of microRNAs.

Material and Methods

Cell Lines and Reagents

All cell lines were purchased from the American Type Culture Collection (Manassas, VA) and maintained in standard media. *PCAT-1* overexpression cell lines were generated using the pLenti6 vector (Invitrogen, Carlsbad, CA), and stably transfected cells were selected by 2.5 μg/ml blasticidin (Invitrogen). *PCAT-1* stable knockdown LNCaP cell lines were transfected with appropriate shRNA lentiviral constructs and a green fluorescent protein (GFP)-positive cell fraction was selected by 1 μg/ml puromycin (Sigma, St Louis, MO). All cell lines were used for fewer than 6 months after resuscitation. Cell lines were confirmed by genotyping and were analyzed by the University of Michigan DNA Sequencing Core using the Profiler Plus PCR Amplification Kit (Applied Biosystems, Foster City, CA). Details on the generation of these isogenic cell lines have been previously published and can be found in reference [13] as well as in the Supplementary Materials.

Cell Proliferation Assays

Cell proliferation assays were performed according to standard protocols using trypan blue staining to enumerate the surviving fraction of cells (see Supplementary Materials).

siRNA Knockdown and MicroRNA Transfection Studies

siRNA knockdowns were performed according to standard protocols (see Supplementary Materials). siRNA sequences (in sense format) for PCAT-1 knockdown were given as follows: siRNA 3 AUACAUAA GACCAUGGAAAU; siRNA 4 GAACCUAACUGGACUUUAAUU. Commercial cMyc siRNAs were obtained from Dharmacon (Dharmacon, Lafayette, CO).

For microRNA transfection, respective microRNA mimics and negative controls were purchased from Ambion (Austin, TX), and transfections were performed with Oligofectamine (Invitrogen) as described previously [14].

Bromouridine Pulse-Chase Labeling Assay

Cells were plated in 100-mm dishes and cultured until the cell confluency reaches 75%. After 30 minutes of bromouridine labeling, followed by uridine chases for 0 and 6 hours, cells were washed with cold phosphate-buffered saline and harvested with TRIzol. Total cell RNA was isolated by a standard protocol, and nascent Bru-labeled mRNA was specifically isolated with anti-bromodeoxyuridine antibodies. Samples were subjected to quantitative polymerase chain reaction (qPCR), and the expression level of target gene was determined. The gene expression levels at 0 and 6 hours represent nascent mRNA synthesis and stability, respectively.

RNA Isolation, cDNA Synthesis, and qPCR Experiments

RNA isolation and cDNA synthesis were performed with TRIzol and Superscript III (Invitrogen), respectively, according to standard protocols (see Supplementary Materials). qPCR was performed with SYBR Green. The primer sequences are listed below: *PCAT-1*-Forward: TGAGAAGAGAAATCTATTGGAACC, *PCAT-1*-Reverse: GGTTTGTC-TCCGCTGCTTTA; *MYC*-Forward: GCTCGTCTCA GAGAAGCTGG, *MYC*-Reverse: GCTCAGATCCTGCAGGTACAA; *ACTB*-Forward: AAGGCCAACCGCGAGAAG, *ACTB*-Reverse: ACAGCCTGGATAGCAACGTACA. Additional primer pair sequences can be found in Table W9.

Immunoblot Analysis

With or without treatment, cells were harvested and lysed in RIPA lysis buffer (Sigma) supplemented with protease inhibitor cocktail (Roche, Basel, Switzerland). Western blot analysis was performed with standard protocols as described before [14,15]. The protein band

densitometry was determined using the image analysis software package Image J (version 1.42, available from the Research Services Branch of NIH).

Luciferase Reporter Assays

The cMyc 3′UTR was amplified using reverse transcription–PCR from LNCAP prostate cancer cells and subcloned into the pRL-TK vector. DU145 control, DU145–*PCAT-1*, RWPE-LacZ, RWPE–*PCAT-1* pool, LNCaP sh–NT, LNCaP sh–*PCAT-1*–#1, and LNCaP sh–*PCAT-1*–#2 were plated onto 24-well plates and transfected with cMyc 3′UTR luciferase constructs as well as pRL-TK vector as internal control for luciferase activity. After 48 hours of incubation, luciferase reporter assay were conducted with the dual luciferase assay kit (Promega, Madison, WI).

Gene Expression Microarrays

Expression profiling was performed using the Agilent Whole Human Genome Oligo Microarray (Santa Clara, CA), according to previously published protocols. All samples were run in technical duplicates comparing RWPE and Du145 cells overexpressing *PCAT-1* and treated with either non-targeting siRNAs or siRNAs targeting cMyc. Expression array data were processed to yield log-2 expression values. Genes that were at least >1.5-fold changed in one cell line and at least >0.5-fold changed in the second cell line were considered to be significantly upregulated or downregulated. All cell line replicates were considered. For gene ontology analysis, upregulated and downregulated probes were separated and probe lists were analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) bioinformatics platform with the functional annotation clustering tool. See the Supplementary Materials for additional details.

Statistical Analyses for Experimental Studies

All data are expressed as means ± SD or SEM. All experimental assays were performed in duplicate or triplicate. Statistical analysis was performed by two-sided Student's *t* test and one-way analysis of variance (ANOVA) with a Student-Newman-Keuls follow-up test. Significance was designed as "*" when $P < .05$.

Results

cMyc Is Required for PCAT-1–Mediated Cell Proliferation

To establish a role for *PCAT-1* in prostate cancer, we overexpressed *PCAT-1* in Du145 prostate cancer and RWPE benign immortalized prostate cells (Figure W1*A*). We further used two shRNA constructs targeting *PCAT-1* to deplete endogenous *PCAT-1* levels in the LNCAP prostate cancer cell line (Figure W1*A*). Additional details on the characterization of these cell lines can be found in reference [13]. We found that overexpression of *PCAT-1* substantially increased cell proliferation in Du145 and RWPE cells, whereas *PCAT-1* knockdown in LNCAP cells reduced cell proliferation (Figure W1, *B*–*D*). We further confirmed that knockdown of *PCAT-1* in Du145–*PCAT-1* and RWPE–*PCAT-1* cells reversed this cell proliferation phenotype, supporting the specificity of our overexpression models (Figure W2). Finally, we confirmed that our *in vitro* models expressed *PCAT-1* at a level similar to endogenous expression by comparing our models with *PCAT-1* expression from a previously published cohort [10] of prostate cancer tissues (Figure W3).

We next sought to describe a mechanistic basis for the role of *PCAT-1* in cell proliferation. To this end, we noted that *PCAT-1* is located on chr8q24, just 725 kb upstream of the *MYC* oncogene (Figure 1*A*, *left*). Given this close proximity, we hypothesized that *PCAT-1* may regulate *MYC* to achieve its functions. Indeed, the *cis*-regulation of neighboring protein-coding genes is a common mechanism for numerous lncRNAs, including the well-studied lncRNAs such as H19 and Xist [5]. In cancer, the *cis*-regulation of neighboring protein-coding genes by lncRNAs has also been implicated in oncogenic processes such as cell invasion [16].

Surprisingly, we found that cMyc protein levels were dramatically increased by *PCAT-1* overexpression in Du145 and RWPE (Figure 1*A*, *right*). Consistent with these observations, knockdown of *PCAT-1* in LNCAP cells reduced cMyc protein (Figure 1*A*, *right*). To verify this relationship, we next performed siRNA knockdown of *PCAT-1* in Du145–*PCAT-1* and RWPE–*PCAT-1* cells and observed a substantial down-regulation of the cMyc protein, confirming the specificity of *PCAT-1*–mediated up-regulation of cMyc (Figure 1*B*). To determine whether cMyc up-regulation played a role in *PCAT-1*–mediated pathogenesis, we performed siRNA knockdown of cMyc in *PCAT-1*–overexpressing cell lines. We found that cMyc knockdown fully abrogated the proliferative effect of *PCAT-1* overexpression in Du145 and RWPE (Figure 1, *C* and *D*). *PCAT-1*–mediated cell proliferation is thus dependent on cMyc overexpression.

Identification of a PCAT-1–cMyc Regulatory Gene Expression Program

We next sought to define downstream effectors of *PCAT-1* and cMyc through the analysis of gene expression changes following modulation of these factors. Specifically, to evaluate whether cMyc stabilization was necessary for *PCAT-1*–mediated gene expression signatures, we performed gene expression array analysis of Du145–*PCAT-1* and RWPE–*PCAT-1* cells treated with cMyc or non-targeting siRNAs and compared them to control RWPE and Du145 cells lacking *PCAT-1*. We confirmed the microarray reproducibility by running each sample in duplicate (Figure W4*A*). Next, we observed a significant overlap ($P < .05$ by Fisher exact test) for probes differentially regulated with *PCAT-1* overexpression in RWPE and Du145 (Figure W4*B*). Using this set of probes, we defined a signature of 184 genes that were significantly upregulated or downregulated by *PCAT-1* and whose expression change was abrogated by cMyc knockdown (Figure 2*A* and Table W1). Analysis of 91 genes downregulated by *PCAT-1* and dependent on cMyc using the DAVID bioinformatics platform revealed enrichment for cytochrome *P*450 genes, which are known downregulated targets of cMyc (Table W2) [17–19]. Conversely, analysis of 93 genes upregulated by *PCAT-1* and dependent on cMyc demonstrated an enrichment of genes involved in protein biosynthesis and transcriptional elongation, well-described functions of cMyc (Table W3) [20,21]. This included both ribosomal subunits such as *RPL9* and *RPLP0* as well as the translation initiation factors *EIF4B* and *EEF1A2*. Interestingly, *PCAT-1* also required cMyc for the down-regulation of several well-established tumor suppressor genes such as *CDKN2B*, a known cMyc target [22,23], as well as *TP73* and *IKZF2*. Together, these data support a role for cMyc in the control of cancer-specific gene expression signatures mediated through *PCAT-1*.

Post-Transcriptional Regulation of cMyc by PCAT-1

To establish a mechanistic basis for cMyc overexpression by *PCAT-1*, we next assessed *MYC* mRNA levels in our *in vitro* models. We found that *MYC* mRNA levels were unchanged in our LNCaP, Du145, and

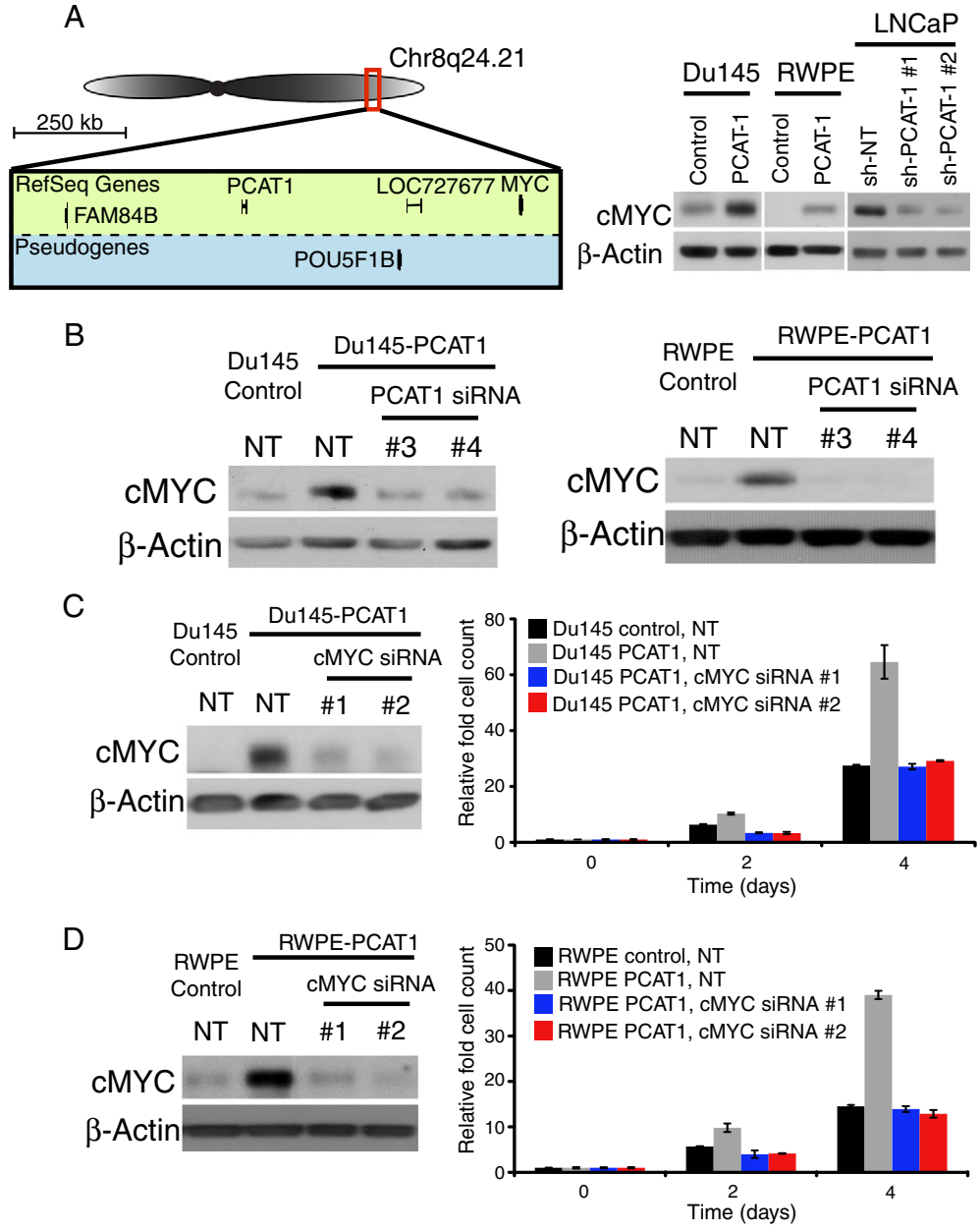

Figure 1. *PCAT-1* mediates cell proliferation through up-regulation of cMyc. (A) Left: A schematic demonstrating the genomic location of *MYC* and *PCAT-1* on chr8q24. *PCAT-1* is approximately 750 kb upstream of *MYC*. Right: Western blot analysis correlating cMyc protein levels with *PCAT-1* expression. cMyc is upregulated with *PCAT-1* overexpression and downregulated with *PCAT-1* knockdown. (B) Left: Knockdown of *PCAT-1* in Du145–*PCAT-1* cells decreases cMyc protein abundance. Right: Knockdown of *PCAT-1* in RWPE–*PCAT-1* cells decreases cMyc protein abundance. (C) Left: Western blot analysis demonstrating efficiency of cMyc knockdown in Du145–*PCAT-1* cells. Right: Cell proliferation of Du145–*PCAT-1* cells with and without cMyc knockdown. (D) Left: Western blot analysis demonstrating efficiency of cMyc knockdown in RWPE–*PCAT-1* cells. Right: Cell proliferation of RWPE–*PCAT-1* cells with and without cMyc knockdown. Error bars represent mean ± SEM. In this figure, NT stands for non-targeting control.

RWPE cell lines (Figure 2*B*). To further examine the regulation of cMyc, we performed bromouridine pulse-chase labeling assays to test the effect of *PCAT-1* on the synthesis and stability of *MYC* mRNA. Similarly, we found no change in *MYC* RNA stability in our model systems (Figure 2*C*). Taken together, these data argue that the regulation of cMyc by *PCAT-1* operates at the post-transcriptional level.

Post-transcriptional regulation of mRNAs is frequently coordinated through the activity of microRNAs binding to the 3′UTR [24,25]. Specifically, increased activity of the 3′UTR can result in increased protein abundance and gene activation. To test whether *PCAT-1* regulated *MYC* through its 3′UTR, we transfected *MYC* 3′UTR luciferase constructs into our isogenic cell lines. Supporting a role in post-transcriptional regulation, we found that *PCAT-1* overexpression increased *MYC* 3′UTR activity, whereas knockdown of *PCAT-1* decreased *MYC* 3′UTR activity (Figure 2*D*).

MicroRNA-Mediated Cross Talk between MYC and PCAT-1

Recently, several groups have argued that a subset of lncRNAs may operate as coordinators of microRNA activity by disrupting microRNA regulation of mRNA 3′UTRs [26–28]. lncRNAs may

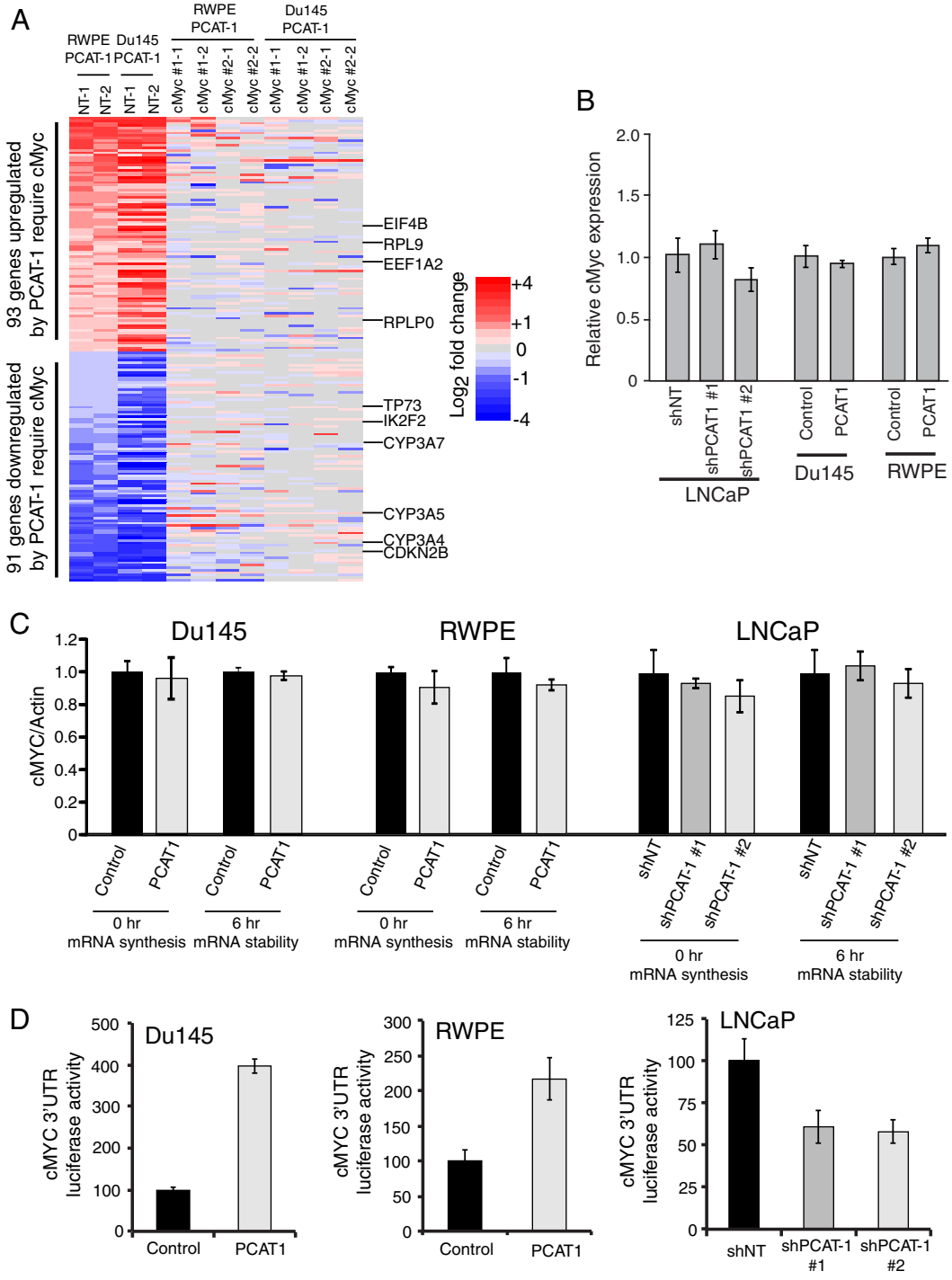

Figure 2. *PCAT-1* regulates cMyc in a post-transcriptional manner by 3′UTR activation. (A) Gene expression analysis of Du145–*PCAT-1* and RWPE–*PCAT-1* cells treated with non-targeting or cMyc siRNAs reveals a gene signature of genes co-regulated by *PCAT-1* and cMyc. In this figure, the columns on the far left show gene expression changes in RWPE–*PCAT-1* and Du145–*PCAT-1* cells treated with non-targeting siRNA compared to RWPE and Du145 control cells treated with non-targeting siRNA, respectively. On the right, RWPE–*PCAT-1* and Du145–*PCAT-1* cells were treated with two independent siRNAs for cMyc, and the indicated gene expression values of these cells were calculated relative to the RWPE and Du145 control cells treated with non-targeting siRNA. All experiments were analyzed with duplicate microarrays, as indicated at the top of the heat map. (B) *MYC* mRNA expression levels are unchanged in three *in vitro* model systems of *PCAT-1*. (C) *MYC* mRNA stability is unchanged in Du145–*PCAT-1*, RWPE–*PCAT-1*, or LNCaP sh–*PCAT-1* cells. (D) Luciferase signal for the *MYC* 3′UTR activity is increased in Du145–*PCAT-1* and RWPE–*PCAT-1* cells but decreased in LNCaP sh–*PCAT-1* cells. In this figure, NT stands for non-targeting control.

thus interfere with microRNA function. Conversely, microRNAs that target lncRNAs would be predicted to titrate away the effect of the lncRNAs. To apply this model to *PCAT-1* regulation of cMyc, we employed four microRNA-target prediction algorithms (miRanda, miRBase, TargetScan, and PicTar) to nominate microRNAs that target *MYC* or *PCAT-1* (Tables W4–W7). For *MYC*,

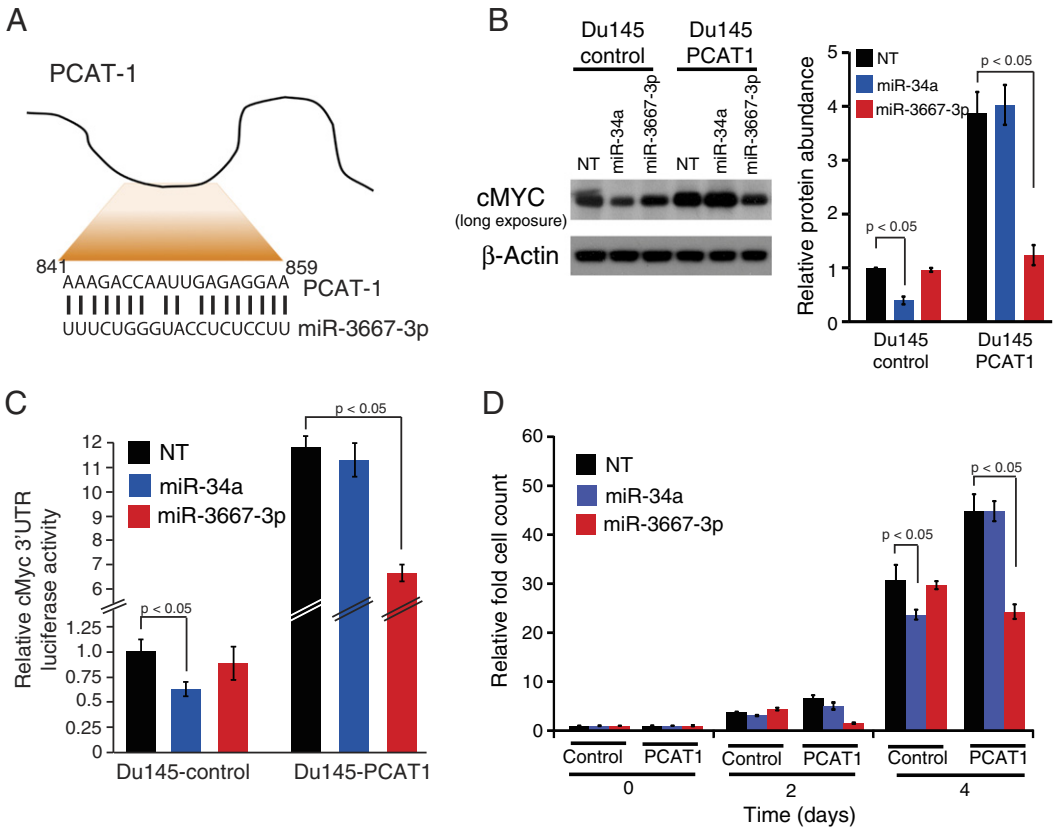

Figure 3. *PCAT-1* interferes with microRNA networks regulating cMyc. (A) A schematic demonstrating base-pair complementation between *PCAT-1* and miR-3667-3p. (B) Left: cMyc protein abundance in Du145 control and Du145–*PCAT-1* cells following overexpression of miR-34a or miR-3667p. Note that a long exposure of the immunoblot is shown to illustrate relative protein abundance. Right: Quantification of cMyc protein abundance. Significance is determined by three independent experiments. (C) *MYC* 3′UTR luciferase signal following overexpression of miR-34a or miR-3667-3p in cells with and without *PCAT-1* overexpression. (D) Cell proliferation of Du145 control or Du145–*PCAT-1* cells following overexpression of miR-34a or miR-3667-3p. A non-targeting NT scrambled RNA serves as a control. Error bars represent mean ± SEM. Statistical significance is determined by Student's *t* test. In this figure, NT stands for non-targeting control.

we recovered known microRNA regulators such as the miR-34 family (including miR-34a, miR-34b, and miR-34c), which has been previously reported to downregulate *MYC* in multiple prostate cancer model systems [29–31]. For *PCAT-1*, we surprisingly found only one microRNA, miR-3667-3p, that was predicted to form high-confidence complementary base pairing with *PCAT-1* (Figure 3*A*).

To assess the effect of *PCAT-1* on microRNA signaling, we overexpressed miR-34a, a known regulator of *MYC*[29], and miR-3667-3p, which was nominated as a *PCAT-1* regulator, in Du145 control and Du145–*PCAT-1* cells. We predicted that *PCAT-1* should antagonize the effects of miR-34a, preventing its regulation of cMyc; conversely, miR-3667-3p should antagonize *PCAT-1* and impact cMyc levels only in *PCAT-1*–overexpressing cells. When tested in our *in vitro* models, we observed that miR-34a appropriately decreased cMyc abundance in Du145 control cells but not in Du145–*PCAT-1* cells (Figure 3*B*). This suggests that *PCAT-1* expression exerts a protective effect on cMyc by interfering with microRNA-based regulation. By contrast, overexpression of miR-3667-3p decreased cMyc protein levels exclusively in Du145–*PCAT-1* cells (Figure 3*B*). Quantification of cMyc protein abundance from these experiments confirmed the significance of these results (Figure 3*B*, *right*). We further confirmed these phenomena in RWPE *PCAT-1* overexpression cells, where miR-34a and miR-3667-3p overexpression produced highly concordant results (Figure W5). We also assessed the endogenous levels of miR-34a and miR-3667-3p in both parental cell lines and overexpression lines (Figure W6).

To confirm that this regulation of cMyc was post-transcriptional, we verified that overexpression of miR-34a and miR-3667-3p specifically decreased the *MYC* 3′UTR activity in Du145 control and Du145–*PCAT-1* cells, respectively (Figure 3*C*). Further, we reasoned that if miR-3667-3p affects cMyc protein abundance by targeting *PCAT-1*, then *PCAT-1* mRNA levels should be depleted on overexpression of this microRNA. Indeed, we found that *PCAT-1* mRNA levels decreased approximately 50% when miR-3667-3p was overexpressed in Du145–*PCAT-1* cells (Figure W7). Finally, to demonstrate the functional impact of miR-34a and miR-3667-3p, we performed cell proliferation assays. Consistent with our model, miR-34a significantly decreased proliferation only in Du145 control cells, whereas miR-3667-3p decreased proliferation only in Du145–*PCAT-1* cells (Figure 3*D*). Analyzing the data shown in Figure 3, *B* to *D*, for statistical significance using a one-way ANOVA test further confirmed a significant P value $<.05$ for all experiments (Table W8). Together,

these data support a model in which *PCAT-1* interferes with the regulation of *MYC* by microRNAs (e.g., miR-34a), thereby increasing cMyc protein levels and enhancing cell proliferation (Figure W8). Targeting of *PCAT-1* by miR-3667-3p is able to reverse its effects by depleting *PCAT-1* and preventing it from upregulating *MYC* 3′ UTR activity and protein abundance.

Discussion

Taken together, this study represents the first analysis of lncRNA-based regulation of cMyc in prostate cancer. We find that *PCAT-1* post-transcriptionally upregulates cMyc protein, leading to cell proliferation and specific gene expression programs, including increased expression of cMyc target programs such as protein translation. Moreover, we investigate a post-transcriptional regulatory web in which *PCAT-1* disrupts microRNA-based regulation of cMyc. Specifically, we find that *PCAT-1* is able to abrogate the down-regulation of cMyc by miR-34a. By contrast, targeting *PCAT-1* with miR-3667-3p is able to reverse the stabilizing effect of *PCAT-1* on cMyc protein levels. These findings correlate with the 3′UTR activity of *MYC*, which is also regulated by *PCAT-1*.

cMyc is a well-studied regulator of cell proliferation and is frequently dysregulated in prostate cancer, where amplification of the cMyc locus occurs in >20% of patients with localized disease and >50% of patients with metastatic disease [32]. cMyc is a ubiquitous transcription factor essential for cell cycle progression, and its oncogenic up-regulation results in wide-ranging consequences on cancer cell biology [33]. Indeed, it is estimated that up to one third of all human genes may be regulated by cMyc in certain contexts [34]. Numerous mechanisms of cMyc regulation have been described in cancer, including the post-transcriptional regulation of cMyc by microRNAs. In particular, miR-34a has been shown to target the 3′UTR of *MYC*, thereby degrading cMyc protein and strongly suppressing cancer cell proliferation and transformation [29,35]. However, regulation of cMyc by prostate cancer lncRNAs has not been previously studied.

In addition, these data give insight into the complex web of post-transcriptional regulation of RNA and protein species in the cell cytoplasm. Here, our work derives from the theory of competing endogenous RNAs proposed by Pandolfi and colleagues [36]. According to this model, cytoplasmic RNA species are able to "titrate away" the presence of other regulatory entities, particularly microRNAs but also pseudogenes and other mRNAs [26–28]. This occurs through competitive disruption of microRNA binding to target genes. Characterization of the *PTEN* mRNA by Pandolfi and colleagues has led to an increased appreciation for the numerous cytoplasmic interactions that may occur at the RNA level and contribution to overall protein abundance and production [36]. We propose that *PCAT-1* functions through a similar web in prostate cancer to coordinate increased cMyc abundance (Figure W8).

With respect to mechanism specifically, we have previously suggested that *PCAT-1* may interact with the nuclear polycomb repressive complex 2 (PRC2) [10]. While a subset of *PCAT-1* transcripts appear to localize to the nucleus, possibly engaging PRC2 for functional effects, we subsequently found that a majority of *PCAT-1* transcripts are located in the cytoplasm [13] and thus likely operates independently of PRC2 in this cellular compartment. This current manuscript therefore supports a function of *PCAT-1* in the cytoplasm but does not exclude potential mechanisms for gene expression regulation in the nucleus through PRC2 or other means. One additional notable point is that we previously showed that *PCAT-1* expression does not correlate well with *MYC* mRNA expression in human prostate cancer tissues. Our current work would support that observation: we find no effect of *PCAT-1* on *MYC* mRNA. In this regard, our work is consistent with evidence showing that microRNA-based regulation can occur primarily through translational inhibition in certain situations [37].

We have also independently shown that *PCAT-1* downregulates BRCA2 [13], and a recent study in breast cancer cells demonstrated that cMyc could downregulate BRCA2 through genomic binding to, and up-regulation of, miR-1245 [38]. However, given the well-documented cell type–specific functions of both microRNAs and cMyc [39–42], the relationship between miR-1245 and cMyc in prostate cells remains an unexplored area of research.

Further, we have observed that *PCAT-1* is able to interfere with the regulation of *MYC* by miR-34a. However, we have been unable to locate a direct binding site for miR-34a in the *PCAT-1* gene sequence. Our analyses are limited by the capabilities of microRNA target prediction software packages, which almost exclusively include protein-coding genes only. miRBase, in fact, was the only software that allowed us to search for microRNAs targeting *PCAT-1* directly. Thus, the effect of *PCAT-1* on miR-34a–mediated regulation may be indirect and may reflect a broader function of *PCAT-1* in which *PCAT-1* disrupts microRNA function indirectly. Here, we are intrigued by the presence of numerous repeat elements in *PCAT-1*, including retroviral long-terminal repeats and an Alu element (see [10]). Repeat elements are known to have effects on microRNA regulation and function [43–45], which may mean that *PCAT-1* is able to impact microRNAs in this manner as well.

More broadly, our work gives insight into role of the 8q24 chromosomal locus in tumor aggressiveness. The 8q24 locus has been studied at length given its frequent amplification in cancer [46], including prostate cancer [32]. While *MYC* is a presumed target for this amplification, it is also clear that the function of 8q24 amplification in cancer is more expansive than simply *MYC* amplification. This is supported by the fact that 8q24 aberrations are typically very large or arm-level amplifications. Indeed, additional targets such as *NCOA2* and *PVT1* have been proposed [47,48]. However, to date, no model has been able to fully elucidate the role of 8q24 amplification in cancer. Here, we suggest that *PCAT-1* may also contribute to the role of 8q24 amplification by augmenting the function of cMyc. This function of *PCAT-1* may support a role for other 8q24 ncRNAs in cancer pathogenesis.

Clinically, *PCAT-1* may be an attractive candidate for biomarker development given its substantial overexpression in prostate cancer and functional role in cancer proliferation. To this end, *PCAT-1* overexpression has been implicated in the poor prognosis of lethal colorectal cancers [12], and *in situ* hybridization assays have been suggested as a possible avenue for the clinical translation of lncRNAs [11]. While the direct therapeutic targeting of lncRNAs has not yet been proven to date, RNA-based therapies are increasingly investigated as potential therapeutic agents. Ultimately, disease-specific targeted therapies against both proteins and lncRNAs may improve the clinical management of refractory prostate cancer.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neo.2014.09.001.

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