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Prostate Cancer

Multi-institutional Analysis Shows that Low *PCAT-14* Expression Associates with Poor Outcomes in Prostate Cancer

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Abstract

Background: Long noncoding RNAs (lncRNAs) are an emerging class of relatively underexplored oncogenic molecules with biological and clinical significance. Current inadequacies for stratifying patients with aggressive disease presents a strong rationale to systematically identify lncRNAs as clinical predictors in localized prostate cancer.
Objective: To identify RNA biomarkers associated with aggressive prostate cancer.
Design, setting, and participants: Radical prostatectomy microarray and clinical data was obtained from 910 patients in three published institutional cohorts: Mayo Clinic I ($N = 545$, median follow-up 13.8 yr), Mayo Clinic II ($N = 235$, median follow-up 6.7 yr), and Thomas Jefferson University ($N = 130$, median follow-up 9.6 yr).
Outcome measurements and statistical analysis: The primary clinical endpoint was distant metastasis-free survival. Secondary endpoints include prostate cancer-specific survival and overall survival. Univariate and multivariate Cox regression were used to evaluate the association of lncRNA expression and these endpoints.
Results and limitations: An integrative analysis revealed *Prostate Cancer Associated Transcript-14* (*PCAT-14*) as the most prevalent lncRNA that is aberrantly expressed in prostate cancer patients. Down-regulation of *PCAT-14* expression significantly associated with Gleason score and a greater probability of metastatic progression, overall survival, and prostate cancer-specific mortality across multiple independent datasets and ethnicities. Low *PCAT-14* expression was implicated with genes involved in biological processes promoting aggressive disease. In-vitro analysis confirmed that low *PCAT-14* expression increased migration while overexpressing *PCAT-14* reduced cellular growth, migration, and invasion.
Conclusions: We discovered that androgen-regulated *PCAT-14* is overexpressed in prostate cancer, suppresses invasive phenotypes, and lower expression is significantly prognostic for multiple clinical endpoints supporting its significance for predicting metastatic disease that could be used to improve patient management.

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Patient summary: We discovered that aberrant *prostate cancer associated transcript-14* expression during prostate cancer progression is prevalent across cancer patients. *Prostate cancer associated transcript-14* is also prognostic for metastatic disease and survival highlighting its importance for stratifying patients that could benefit from treatment intensification.

1. Introduction

The majority of prostate cancer patients are diagnosed at a potentially curable stage and are often treated with radical prostatectomy or other first-line treatments [1]. However, a subset of patients with aggressive disease face the risk of prostate cancer recurrence, which can manifest as persistently elevated/increasing serum prostate-specific antigen or metastasis. Therefore, a critical goal in prostate cancer research is determining the molecular underpinnings of aggressive and indolent disease to improve patient management and prognosis. Recent studies demonstrated the utility of protein-coding genes as prognostic biomarkers [2,3]. However, the lack of tissue specificity hinders the ability to monitor their expression levels noninvasively. In contrast, long noncoding RNAs (lncRNAs) are used successfully to diagnosis and risk stratify prostate cancer [4,5]. Therefore, our study focuses on exploring lncRNAs as biomarkers for risk stratification to ultimately improve patient management.

Our group and others have leveraged next-generation sequencing to generate an unbiased transcriptome to discover novel lncRNAs in prostate cancer [6–8]. This has spurred numerous studies exploring the biological and clinical significance of lncRNAs [5,9,10]. However, to date, all of the transcriptome-based discoveries have utilized patient samples from high quality specimens (ie, abundance of tissue, snap frozen tissue, recently collected), which made them ideal to perform transcriptome sequencing but lacked longer-term clinical outcomes. To address this, we performed an integrative analysis of multiple independent transcriptome data collections [7,11,12] coupled with an unconventional microarray platform, with probe coverage of a significant portion of lncRNAs, to associate lncRNA expression with long-term outcomes such as metastasis, overall survival, and prostate-cancer specific mortality.

2. Patients and methods

2.1. Clinical association of PCAT-14

Microarray data was obtained from four previously published datasets from the Decipher Genomic Resource Information Database [13–16]. Data can also be found in the Gene Expression Omnibus: GSE29079, GSE46691, GSE62116, and GSE72291. Cancer versus normal analysis was performed in the pooled cohorts by Brase et al [13]. Clinical endpoints were evaluated in the Mayo Clinic I and II (MCI and II) cohorts and the Thomas Jefferson University (TJU) cohort. The MCI cohort consisted of 545 patients from a nested case control study with matched triplets for no recurrence, biochemical recurrence only, and metastasis after prostatectomy. The MCII cohort consisted of a case-cohort study with a 20% random sampling from 1010 men with high-risk prostate cancer that was enriched with all of the remaining metastatic cases from the 80% of men not sampled resulting in 235 patients. Postsurgical therapies for both Mayo cohorts were at the treating physician's discretion. The TJU cohort consisted of 130 patients with pT3 or margin-positive disease who received postoperative radiation therapy, with hormone therapy at the treating physician's discretion. *PCAT-14* expression was calculated by taking the mean expression of probe sets mapping to exons. A Student's *t*-test was used to test differences in expression between cancer and normal samples. High/low *PCAT-14* expression was determined by splitting at the median expression level. Kaplan-Meier curves are shown and statistical inference was performed using the Log-rank test. Addition of *PCAT-14* to Cancer of the Prostate Risk Assessment Postsurgical score (CAPRA-S) [17,18] was obtained by training a logistic regression model with both variables, and the model predictions were compared with CAPRA-S alone [5]. Time-dependent receiver operating curves and the area under the curve was calculated using the "timeROC" R package (by Paul Blanche). Multivariable analysis was performed using Cox regression stratified by cohort to adjust for baseline differences. Association of *PCAT-14* with clinicopathologic variables was performed and statistical association with continuous variables was assessed using a Student *t*-test, and categorical variables with the chi-square test. All statistical tests were two-sided and significance was set as $p < 0.05$. Our study adheres to Reporting recommendations for tumor marker prognostic studies criteria [19].

3. Results

3.1. Integrative lncRNA analysis

To identify consistently altered lncRNAs during prostate cancer progression, we performed an integrative analysis of three patient cohorts (Supplementary Table 1): (1) transcriptome sequencing of 14 primary tumors and matched adjacent normal tissue (Ren et al cohort) [7], (2) transcriptome sequencing of 20 primary tumors and 10 matched adjacent normal tissues (Kannan et al cohort) [12], and (3) Affymetrix gene expression of 131 primary and 19 metastatic tumors (Taylor et al cohort) [11]. Four lncRNAs were up-regulated and one lncRNA was down-regulated between the primary tumors and normal tissue across all three cohorts (Fig. 1A, Supplementary Tables 2–5).

Leveraging the clinical data associated with the Taylor et al cohort [11], we next assessed if the lncRNAs were associated with aggressive disease based on Gleason score. While multiple candidates are reported to have altered expression in prostate tumors (ie, *DRAIC* [9], *PCAT-14* [6], *PCAT-101* [6]), *PCAT-14* was the only lncRNA significantly down-regulated in patients with high (9) relative to low (6) Gleason scores ($p = 0.00013$; Fig. 1B, Supplementary Table 2) and negatively correlated with Gleason score (correlation = −0.22). Confirming earlier findings [6], *PCAT-14* displayed altered expression throughout prostate tumor

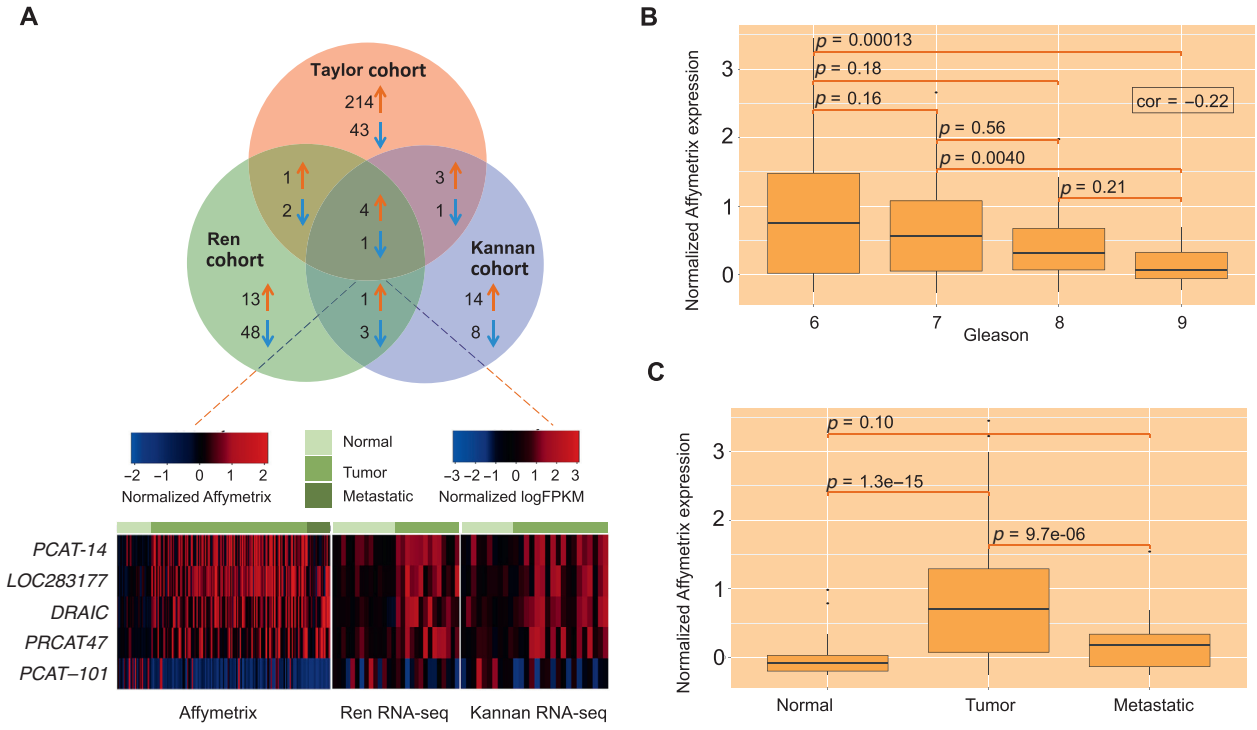

Fig. 1 – Integrative analysis reveals *Prostate Cancer Associated Transcript-14* (*PCAT-14*) expression associates with prostate cancer. (A) Differentially expressed (DE) long noncoding RNAs (lncRNAs) between normal and tumor samples of prostate cancer in one Affymetrix dataset and two RNA-seq datasets (Supplementary Table 1). The Venn diagram shows the number of up-regulated (orange arrow) and down-regulated (blue arrow) DE lncRNAs identified in one to three datasets. Heatmaps of the normalized Affymetrix expression and normalized RNA-seq log fragments per kilobase of exon per million fragments mapped (FPKM) of the five DE lncRNAs from all three datasets are shown across normal, tumor, and metastatic samples in each datasets. Four of the five DE lncRNAs are up-regulated in the tumor dataset and one is down-regulated in all three datasets. Among these five lncRNAs, *PCAT-14* is the only one that shows an association with tumor progression (Supplementary Table 2). (B) Boxplot of normalized Affymetrix expression of *PCAT-14* in Gleason 6 through 9. Expression of *PCAT-14* is anticorrelated with Gleason score (correlation [cor] = −0.22). Expression of *PCAT-14* is significantly decreases from Gleason 6 to 9 ($p = 0.00013$). (C) Boxplot of normalized Affymetrix expression of *PCAT-14* in normal, tumor, and metastatic prostate samples. *PCAT-14* is up-regulated in tumor samples compared with normal samples ($p = 1.3e-15$), and its expression goes down again in metastatic patients from the tumor dataset ($p = 9.7e-06$).

progression (Fig. 1C, Supplementary Fig. 1) as exemplified by *PCAT-14* up-regulation in primary tumors compared with normal tissue ($p = 1.3e-15$) and *PCAT-14* down-regulation in metastatic relative to primary tumors ($p = 9.7e-06$).

Notably, *PCAT-14* expression is enriched in prostate cancer as shown in our pan-cancer expression analysis of 6853 specimens across 18 additional solid tumors, as part of The Cancer Genome Atlas consortium (Supplementary Fig. 2). This is further supported by a recent study reporting *PCAT-14*, under the gene alias *PRCAT-104*, to have altered expression specifically in prostate cancer [8]. Validation of *PCAT-14* expression in a prostate cancer cell line panel relative to the control cell line, RWPE, confirmed over-expression of *PCAT-14* in 22Rv1 and VCaP cell lines (Supplementary Fig. 3A). Subcellular localization revealed that *PCAT-14* is enriched in the nucleus (Supplementary Fig. 3B), which is common amongst lncRNAs associated with gene regulation.

3.2. *Clinical significance of PCAT-14*

Given the altered expression of *PCAT-14* in prostate cancer, we hypothesized a potential relationship with clinical outcomes. Therefore, we assessed *PCAT-14* expression within a cohort of 910 radical prostatectomy specimens from three independent patient cohorts from the Decipher Genomic Resource Information Database: MCI ($N = 545$), MCII ($N = 235$), and TJU ($N = 130$). There were 298 total metastasis events (124 in *PCAT-14* high patients, 174 in *PCAT-14* low patients), 166 total deaths from prostate cancer (69 in *PCAT-14* high patients, 97 in *PCAT-14* low patients), and 366 total deaths (175 in *PCAT-14* high patients, 191 in *PCAT-14* low patients) in our cohorts. Patients with high versus low expression of *PCAT-14* showed significantly different rates of distant metastasis free survival (MCI: $p = 0.0024$, hazard ration [HR] = 0.66 [0.5–0.86], MCII: $p = 0.023$, HR = 0.59 [0.37–0.94], TJU [borderline]: $p = 0.093$, HR = 0.33 [0.084–1.3]) in Fig. 2A, overall survival (MCI: $p = 0.0044$, HR = 0.71 [0.56–0.9], MCII: $p = 0.14$, HR = 0.68 [0.41–1.1], TJU: $p = 0.0061$, HR = 0.35 [0.16–0.77]) in Fig. 2B, and prostate cancer specific survival (MCI: $p = 0.00059$, HR = 0.54 [0.38–0.77], MCII: $p = 0.023$, HR = 0.44 [0.22–0.91], TJU [unavailable]) in Fig. 2C. Consistent with these data, lower *PCAT-14* expression associated with Gleason score as well as lymph node invasion (Table 1). The prognostic ability of *PCAT-14* is significant even after accounting for clinicopathologic variables (age, prostate-specific antigen, Gleason, surgical

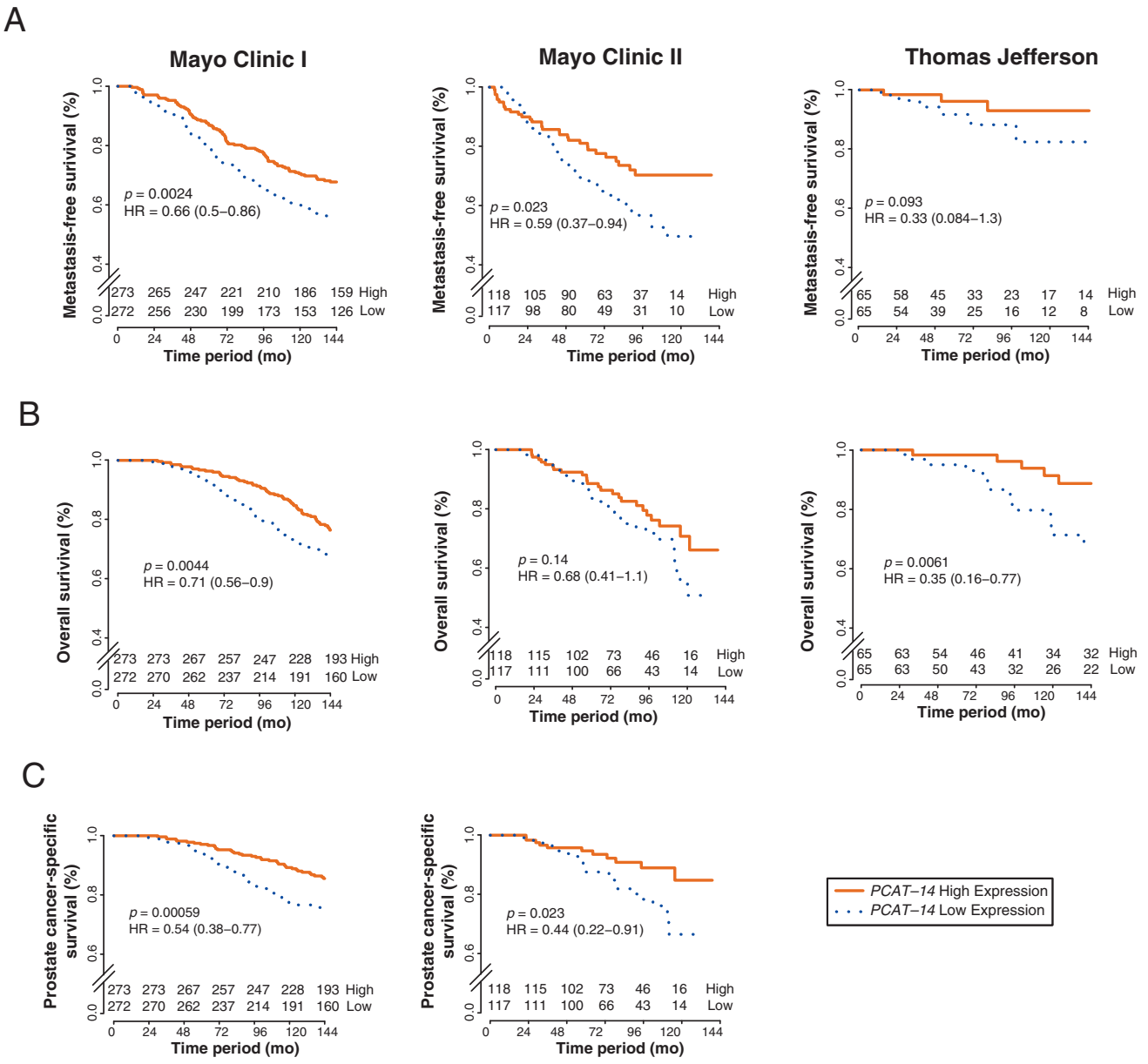

Fig. 2 – *Prostate cancer associated transcript-14* (*PCAT-14*) as a single gene predictor of aggressive disease. Kaplan-Meier analyses of prostate cancer outcomes in the Mayo Clinic cohort. *PCAT-14* expression was measured using Affymetrix exon arrays, and participants were stratified according to *PCAT-14* expression. Participant outcomes were analyzed for (A) distant metastasis-free survival, (B) overall survival, and (C) prostate cancer specific survival, across three patients cohorts (from left to right) Mayo I, Mayo II, and Thomas Jefferson. The *p* values were calculated using a log-rank test. The number at risk is shown at the bottom of each plot.
HR = hazard ratio.

margin status, seminal vesicle invasion, extracapsular extension, and lymph node invasion) on a pooled multivariable cox analysis (Table 2) of these cohorts (distant metastasis free survival: $p = 0.002$, HR = 0.68 [0.53–0.87], prostate cancer specific survival: $p = 0.015$, HR = 0.68 [0.53–0.87], borderline significant for overall survival: $p = 0.056$, HR = 0.81 [0.65–1.01]).

Additionally, we investigated if *PCAT-14* would improve the performance of an existing prediction algorithm, CAPRA-S. We found that when we added *PCAT-14* to CAPRA-S, it increased the receiver operating characteristic area under the curve for 10-yr metastasis rates from 0.68 (CAPRA-S alone) to 0.70 (CAPRA-S + *PCAT-14*, $p = 0.0185$; Supplementary Fig. 4).

3.3. *High PCAT-14 expression promotes a less aggressive phenotype in vitro*

To elucidate the mechanisms of *PCAT-14* function, we utilized our large patient cohort to identify genes with correlated expression profiles to implicate *PCAT-14* in various biological processes (Supplementary Fig. 5). After

Table 1 – Demographics in pooled cohort

	High *PCAT-14*	Low *PCAT-14*	Total	*p* value
Age (yr)	63.8 ± 7.07	64.2 ± 6.91	64 ± 6.99	0.38
Missing	0	0	0	
PSA <10	250 (0.549)	243 (0.534)	493 (0.542)	0.813
PSA 10–20	98 (0.215)	106 (0.233)	204 (0.224)	
PSA >20	95 (0.209)	96 (0.211)	191 (0.21)	
Missing	12 (0.026)	10 (0.022)	22 (0.024)	
Gleason <7	62 (0.136)	37 (0.081)	99 (0.109)	2.63E-10
Gleason =7	268 (0.589)	196 (0.431)	464 (0.51)	
Gleason 8–10	125 (0.275)	221 (0.486)	346 (0.38)	
Missing	0 (0)	1 (0.002)	1 (0.001)	
Margins –	220 (0.484)	190 (0.418)	410 (0.451)	0.0533
Margins +	235 (0.516)	265 (0.582)	500 (0.549)	
Missing	0 (0)	0 (0)	0 (0)	
SVI –	310 (0.681)	292 (0.642)	602 (0.662)	0.234
SVI +	145 (0.319)	163 (0.358)	308 (0.338)	
Missing	0 (0)	0 (0)	0 (0)	
ECE –	224 (0.492)	205 (0.451)	429 (0.471)	0.244
ECE +	230 (0.505)	248 (0.545)	478 (0.525)	
Missing	1 (0.002)	2 (0.004)	3 (0.003)	
LNI –	411 (0.903)	390 (0.857)	801 (0.88)	0.0323
LNI +	43 (0.095)	65 (0.143)	108 (0.119)	
Missing	1 (0.002)	0 (0)	1 (0.001)	

ECE = extracapsular extension; LNI = lymph node invasion; *PCAT-14 = Prostate Cancer Associated Transcript-14*; PSA = prostate-specific antigen; SVI = seminal vesical involvement.

ranking all genes according to their correlation value, we used Gene Set Enrichment Analysis [20] to search for enrichment across the Molecular Signatures Database [21]. We found an enrichment of Lys^{27} of histone 3 methylated target genes among the highest ranked concepts, which were inversely correlated with *PCAT-14* expression (Supplementary Fig. 5). Notably, EZH2 is overexpressed in metastatic prostate cancer, a marker of aggressive disease, and is a critical component of the polycomb repressive complex 2 that methylates Lys^{27} of histone 3 to epigenetically silence target genes associated with metastasis and poor patient outcome [22]. Furthermore, we observed numerous signatures associated with tumor microenvironment that could also be indicative of aggressive disease (Supplementary Fig. 5). Building upon this, and to further support our clinical findings that low *PCAT-14* promotes aggressive disease, we evaluated what effect changes in *PCAT-14* expression had in vitro. Functional significance of *PCAT-14* was assessed in aggressive cell lines, IGR-Cap1 and PC3, with low endogenous expression of *PCAT-14* and well known to metastasize [23–25], by stably overexpressing the full length *PCAT-14* clones.

Our *in vitro* findings support the clinical observations by demonstrating increased *PCAT-14* expression resulted in

Table 2 – Univariate and multivariate analysis

		Univariate		Multivariate	
		p value	HR (95% CI)	*p* value	HR (95% CI)
DMFS	Age	0.76	1 (0.99–1.02)	0.127	0.99 (0.97–1)
	PSA (int vs low)	0.452	1.12 (0.84–1.49)	0.317	0.86 (0.64–1.16)
	PSA (high vs low)	0.00539	1.48 (1.12–1.94)	0.914	0.98 (0.72–1.34)
	Gleason (int vs low)	0.000805	3.69 (1.72–7.93)	0.00651	2.91 (1.35–6.29)
	Gleason (high vs low)	2.70E-09	9.93 (4.66–21.16)	1.62E-06	6.64 (3.06–14.39)
	SMS	0.0616	1.25 (0.99–1.57)	0.775	1.04 (0.81–1.32)
	SVI	6.92E-11	2.14 (1.7–2.69)	0.000659	1.58 (1.21–2.05)
	ECE	5.54E-08	1.93 (1.52–2.45)	0.0854	1.26 (0.97–1.64)
	LNI	5.65E-08	2.16 (1.63–2.85)	0.289	1.18 (0.87–1.62)
	PCAT14 (high vs low)	1.09E-06	0.56 (0.44–0.71)	0.00201	0.68 (0.53–0.87)
PCSS	Age	0.638	1.01 (0.98–1.03)	0.472	0.99 (0.97–1.02)
	PSA (int vs low)	0.386	1.18 (0.81–1.74)	0.108	0.72 (0.49–1.07)
	PSA (high vs low)	0.038	1.47 (1.02–2.11)	0.0349	0.64 (0.43–0.97)
	Gleason (int vs low)	0.0536	2.74 (0.98–7.62)	0.183	2.02 (0.72–5.66)
	Gleason (high vs low)	1.29E-06	11.79 (4.34–32.01)	2.36E-04	6.82 (2.45–18.96)
	SMS	0.000617	1.73 (1.26–2.37)	0.0716	1.35 (0.97–1.87)
	SVI	2.89E-13	3.16 (2.32–4.3)	0.0000276	2.14 (1.5–3.05)
	ECE	1.08E-07	2.42 (1.75–3.36)	0.103	1.34 (0.94–1.92)
	LNI	1.85E-12	3.32 (2.38–4.64)	0.0215	1.57 (1.07–2.29)
	PCAT14 (high vs low)	6.54E-05	0.53 (0.39–0.72)	0.0154	0.67 (0.48–0.93)
OS	Age	1.45E-05	1.04 (1.02–1.06)	0.00541	1.03 (1.01–1.05)
	PSA (int vs low)	0.751	1.04 (0.8–1.36)	0.0548	0.77 (0.58–1.01)
	PSA (high vs low)	0.011	1.38 (1.08–1.78)	0.204	0.83 (0.62–1.11)
	Gleason (int vs low)	0.00256	2.06 (1.29–3.29)	0.0321	1.71 (1.05–2.79)
	Gleason (high vs low)	6.58E-12	5.09 (3.2–8.11)	2.86E-07	3.68 (2.24–6.05)
	SMS	0.000483	1.46 (1.18–1.8)	0.0397	1.26 (1.01–1.57)
	SVI	6.34E-09	1.86 (1.51–2.29)	0.00385	1.43 (1.12–1.83)
	ECE	9.76E-07	1.72 (1.38–2.14)	0.221	1.16 (0.91–1.48)
	LNI	5.38E-08	2.07 (1.59–2.69)	0.0605	1.33 (0.99–1.79)
	PCAT14 (high vs low)	0.00019	0.67 (0.54–0.83)	0.056	0.81 (0.65–1.01)

CI = confidence interval; DMFS = distant metastasis-free survival; ECE = extracapsular extension; HR = hazard ratio; LNI = lymph node invasion; OS = overall survival; *PCAT-14 = Prostate Cancer Associated Transcript-14*; PCSS = prostate cancer-specific survival; PSA = prostate-specific antigen; SMS = surgical margin status; SVI = seminal vesical involvement.

less aggressive phenotypes. In vitro experiments measuring the mobility of cells overexpressing *PCAT-14* show a decreased migratory/invasive capacity of these cells relative to the parental cell line with low *PCAT-14* expression. *PCAT-14* overexpressing IGR-Cap1 cells and empty vector control IGR-Cap1 cells were plated in serum-free media on a transwell membrane and allowed to migrate in a modified Boyden Chamber assay. After 24 h there was a significant 45% decrease in Clone 1 ($p \leq 0.00001$) and 26% decrease in Clone 2 ($p \leq 0.001$) in the migration of *PCAT-14* overexpressing cells compared with control cells (Fig. 3A). This decrease in migration was also seen in PC3 *PCAT-14* overexpressing cells (Fig. 3B). There was at least an 80% decrease in migrated cells in both PC3 *PCAT-14* overexpressing cell lines compared with the control cell line ($p \leq 0.00001$). Further, experiments were conducted with a Matrigel-coated transwell to further confirm altering *PCAT-14* expression changes the aggressiveness of prostate cell lines. Similar to migration assays, *PCAT-14* overexpression in both IGR-Cap1 and PC3 cells significantly diminished cellular invasion compared with the control cell lines (Supplementary Fig. 6). Similarly, silencing *PCAT-14* with a combination of small interfering RNAs (50% knockdown) in 22Rv1 cells with high endogenous expression resulted in a 137% ($p \leq 0.002$) increase in migration relative to control transfected cells (Supplementary Fig. 7). The changes in migratory and invasive cellular behavior highlight that changes in expression levels of *PCAT-14* in prostate cancer cause a less (high expression) or more (low expression) aggressive phenotype. Cellular proliferation was also monitored as an additional characterization of an aggressive phenotype. IGR-Cap1 cells overexpressing two different clones of *PCAT-14* showed a 30% decrease in cell growth relative to control cells at Day 2 ($p = 0.001$ and $p = 0.00001$). The diminished growth in *PCAT-14*-expressing cells continued to Day 6 with a 16% and 37% change in Clone 1 and Clone 2 cell growth, respectively (Supplementary Fig. 8). Combined these data indicate that increased *PCAT-14* expression diminishes oncogenic phenotypes in two well-studied aggressive prostate cancer cell lines; further, silencing *PCAT-14* promotes an aggressive phenotype. Moreover, these data support the strong clinical observations that low *PCAT-14* expression associates with aggressive disease.

3.4. *PCAT-14* association with androgen deprivation therapy

Through Gene Set Enrichment Analysis, we also found that high *PCAT-14* expression had a positive correlation with genes involved in prostate cancer (Supplementary Fig. 5) and androgen response (Fig. 4A). We also observed a moderate correlation (0.49) between *PCAT-14* expression and androgen receptor transcriptional activity (Supplementary Fig. 9). Therefore, we investigated if *PCAT-14* predicted the response to therapies such as androgen deprivation therapy (ADT) in the MCI and MCII cohorts. Of 780 patients, 236 underwent postoperative ADT within 1-yr of radical prostatectomy. We found that the distant metastasis-free survival prognostic differences between *PCAT-14* high and low expression are increased in patients treated with ADT ($p = 0.00082$, HR = 0.5 [0.34–0.76]), and these differences are attenuated in patients without ADT treatment ($p = 0.015$, HR = 0.69 [0.51–0.93]) as shown in Figure 4B. As our cohorts are all retrospective, which confounds treatment by baseline risk, it is necessary to adjust for clinical and pathologic variables and other treatments such as radiotherapy. In our multivariate Cox model, which accounts for these confounders, we found statistically significant interaction terms for ADT and *PCAT-14* ($p = 0.029$) indicating that *PCAT-14* can potentially predict the response to ADT after prostatectomy (Supplementary Table 6). This is supported by *PCAT-14* responsiveness to testosterone and R1881 in VCaP cells (Supplementary Fig. 10). After androgen deprivation, VCaP cells were treated with either 10 nM testosterone or 5 nM of R1881. There was a six-fold and approximately four-fold increase in *PCAT-14* expression with testosterone and R1881 treatment, respectively, relative to no treatment control. As a positive control, there was also a robust increase in *TMPRSS2* gene expression with both drug treatments.

4. Discussion

Our integrative analysis led us to discover a subset of lncRNAs consistently altered across prostate cancer patients suggesting their significance in prostate cancer etiology. Notably, the Ren et al cohort [7] is comprised of Chinese patients suggesting that these five lncRNAs are altered independent of ethnicity. The importance of these lncRNAs is exemplified by a recent study showing that *DRAIC* plays a tumor-suppressive role, displays decreased expression in prostate cancer cell progression from androgen-dependent to a castration-resistant state, and inhibits cellular migration and invasion [9]. Less is known about the remaining candidates that were only recently discovered and reported to have altered expression during prostate tumorigenesis [6,8] or are not yet associated with prostate cancer (ie, *LOC283177*). However, *PCAT-14* was the only candidate that showed an inverse relationship with Gleason score suggestive of its role in suppressing aggressive disease, and therefore represents an ideal candidate to investigate.

Herein, we show that *PCAT-14* is overexpressed in prostate tumors and that lower *PCAT-14* expression associates with three clinical endpoints (distant metastasis-free survival, prostate cancer specific survival, and overall survival). Supporting our clinical findings, we have provided experimental data highlighting the biological role of *PCAT-14* in prostate cancer. Firstly, using patient data we were able to associate low *PCAT-14* expression with biological concepts associated with aggressive disease. Secondly, we demonstrated that *PCAT-14* suppresses metastatic phenotypes in vitro and appears to lose expression in metastatic samples. Stably overexpressing *PCAT-14* in both IGR-Cap1 and PC3 cells resulted in significantly less cellular mobility relative to respective control cell lines. Moreover, the opposite effect—a more oncogenic phenotype—was observed in *PCAT-14* silenced 22Rv1 cells. Taken together, this represents the first study

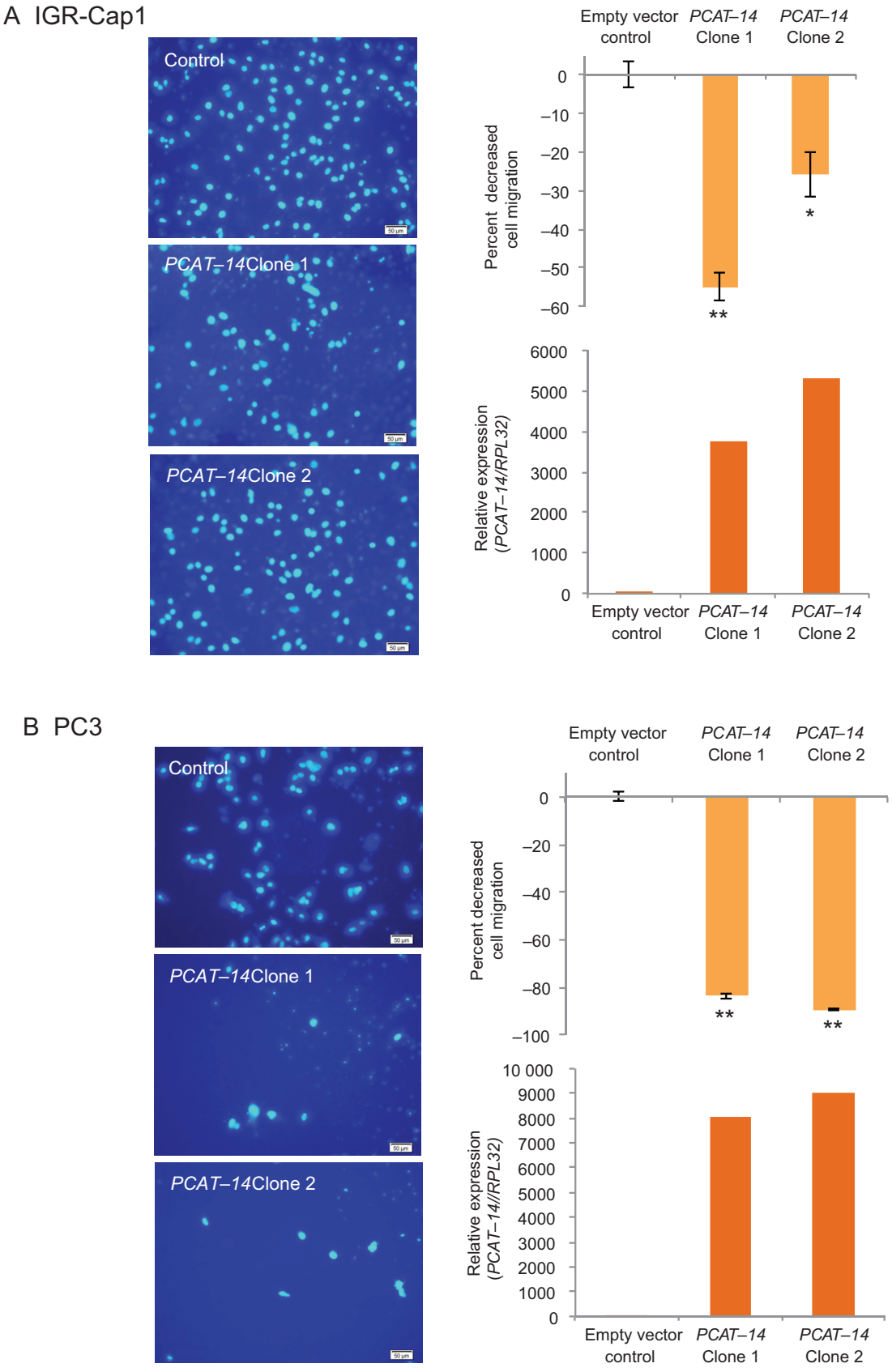

Fig. 3 – Overexpression of *Prostate Cancer Associated Transcript-14* (*PCAT-14*) diminishes cellular migration in vitro. (A) IGR-Cap1 or (B) PC3 cells were plated for 24 h on a transwell membrane. Migrated cells (bottom of filter) were stained with 4′,6-diamidino-2-phenylindole and quantified. Quantitative reverse transcription polymerase chain reaction confirmed the overexpression of *PCAT-14* in both cell lines relative to empty vector control cell lines.

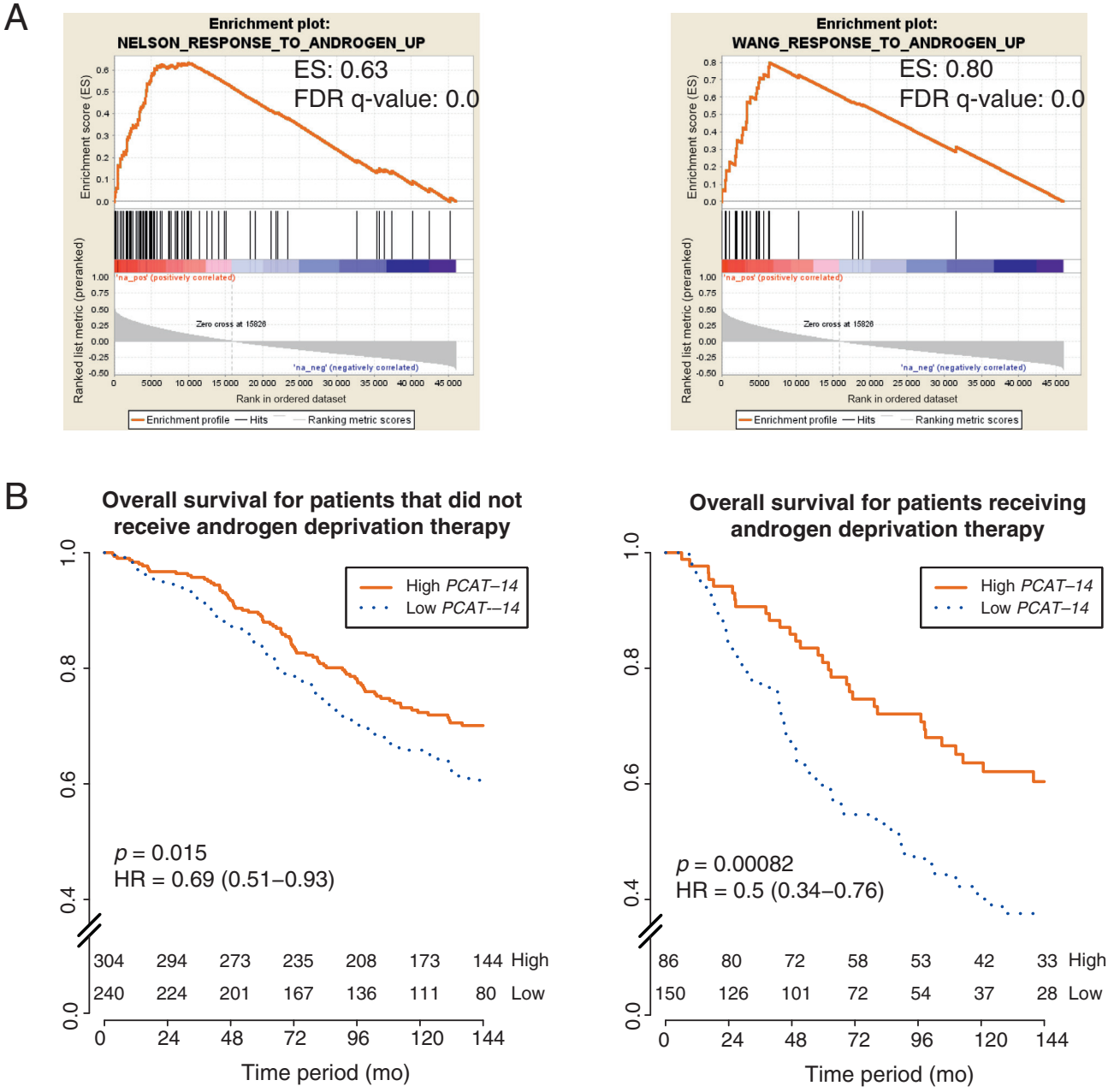

Fig. 4 – *Prostate Cancer Associated Transcript-14* (*PCAT-14*) is predictive of androgen deprivation therapy response. (A) Gene set enrichment analysis of genes coexpressed with *PCAT-14* revealed gene signatures associated with androgen response. Enrichment Score (ES) and false discovery rate (FDR) q-value are shown within the gene set enrichment analysis plot. (B) Kaplan-Meier curves show that high *PCAT-14* expression is differentially prognostic of distant metastasis-free survival in patients treated with (left) and without (right) androgen deprivation therapy.
HR = hazard ratio.

highlighting the biological significance of *PCAT-14* in prostate cancer as well as the clinical significance of low *PCAT-14* expression for predicting outcome.

Notably, we observed that patients who received ADT appeared to have a greater distant metastasis-free survival prognostic difference between *PCAT-14* high and low expression in comparison to patients that did not receive ADT. As these are retrospective studies, there are inherent differences between patients that received treatment and those that did not receive treatment. However, our multivariate interaction analysis coupled with the in vitro data demonstrating that *PCAT-14* is androgen responsive suggests that *PCAT-14* warrants further study as a predictive marker for ADT response.

5. Conclusions

Overall, we discovered that *PCAT-14* is commonly up-regulated in primary tumors. Furthermore, low *PCAT-14* expression promotes aggressive oncogenic phenotypes and

is significantly prognostic for multiple clinical endpoints. In addition to predicting metastatic disease, we found that *PCAT-14* may be able to predict ADT response highlighting its potential use for improving patient management and prognosis.

Author contributions: Christopher A. Maher had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: White, Zhao, Maher.
Acquisition of data: Zhao, Zhang, White, Alshalalfa, Erho, Rozycki, McFadden, Dang, Karnes, Den, Davicioni.
Analysis and interpretation of data: White, Zhao, Zhang, Dang, Alshalalfa, Vergara, Erho, Eteleeb, Maher.
Drafting of the manuscript: White, Zhao, Maher.
Critical revision of the manuscript for important intellectual content: White, Zhao, Maher.
Statistical analysis: Zhao, Alshalalfa, Vergara, Erho, Zhang.
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Supervision: Maher.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2016.07.012.

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