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**14. ABSTRACT**

In the last year, we made significant progress in determining the role of microRNAs in driving neuroendocrine prostate cancer (NEPC).

We demonstrated that progression of advanced CRPC with adenocarcinoma characteristics (CRPC-Adeno) to therapy-induced, androgen-independent NE (CRPC-NE) states is associated with a characteristic set of miRNA alterations that promote plasticity of advanced prostate adenocarcinomas to NEPC (Bhagirath et al., Oncogene, 2020). Importantly, we could develop a 'novel miRNA classifier' to robustly stratify CRPC-NE tumors from CRPC-Adenocarcinomas. Further validation of the classifier in clinical samples from two independent sites showed a '5-miRNA' classifier to be of significance in distinguishing between CRPC-Adenocarcinomas and CRPC-NE tumors. This classifier included downregulation of miR-28-3p as an important feature. In view of this data, we examined the functional role of miR-28-3p in prostate cancer. Our studies suggest that miR-28-3p plays a tumor suppressive role in advanced prostate cancer. We discovered that this miRNA directly represses Vimentin, a mesenchymal gene. Therefore, loss of this miRNA in advanced prostate cancer leads to the upregulation of Vimentin and induction of epithelial-to-mesenchymal transition (EMT), favoring tumor progression. Furthermore, this miRNA represses oncogenic Akt3-mediated signaling. In addition, we performed *in vivo* studies on miR-410 in prostate cancer. These studies demonstrated an oncogenic role of miR-410 in neuroendocrine prostate cancer.

**15. SUBJECT TERMS**

MicroRNAs, neuroendocrine differentiation, castration-resistant prostate cancer

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## TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research*

The objective of this project is to define the miRNA alterations associated with progression of advanced castration-resistant prostate cancer (CRPC) to androgen-independent neuroendocrine prostate cancer (NEPC). NEPC is an extremely aggressive variant of CRPC that emerges upon highly potent androgen receptor (AR)-targeted therapies such as Enzalutamide (ENZ) and Abiraterone (ABI) that is increasing in incidence. This variant arises via a reversible trans-differentiation process known as neuroendocrine differentiation (NED), wherein prostate cancer cells undergo a lineage switch with altered expression of lineage markers such as decreased expression of androgen receptor (AR) and increased expression of alternative neuroendocrine (NE) lineage markers including enolase 2 (ENO2), chromogranin A (CHGA) and synaptophysin (SYP). The mechanistic basis of NED is poorly understood contributing to the lack of effective molecular markers for prediction and late recognition of the disease. With a goal of understanding molecular mechanisms underlying the transition of CRPC to NEPC and resistance to AR pathway inhibitors, we are aiming to characterize the significant microRNA (miRNA) alterations associated with progression of advanced CRPC with adenocarcinoma characteristics (CRPC-Adeno) to androgen-independent neuroendocrine state (CRPC-NE). Though the genetic and epigenetic basis of NEPC has been investigated previously, miRNA alterations associated with NEPC and their associated roles have not been systematically investigated yet.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

MicroRNAs, neuroendocrine differentiation, castration-resistant prostate cancer

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

**What were the major goals of the project?**

*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

The major goal of the project is to understand the miRNA alterations that drive NED in advanced CRPC. The underlying hypothesis is that NED is associated with significant alterations to the miRNAome, that in turn, drives change in cellular gene expression patterns towards NE states. We proposed to test our hypothesis under the following specific aims:

**Specific Aim 1**: **Examine the dysregulated microRNA repertoire of neuroendocrine prostate cancer.** We proposed to perform small RNA sequencing in (i) cells from '*in vitro'* induced models of NEPC (ii) microdissected PCa clinical tissues from CRPC-Adeno (castration-resistant prostate cancer with adenocarcinoma features) vs CRPC-NE (CRPC with NE features).

**Specific Aim 2**: **Determine the functional role of top dysregulated miRNAs in NED in prostate cancer.** Under this specific aim, we proposed to examine the functional role of two significantly downregulated miRNA clusters/miRNAs in NEPC (miR-410, miR-17/92 cluster and/or miR-363) that were identified by NGS.

**Specific Aim 3: Determine the prognostic potential of miRNAs regulating NEPC.** We propose that NE- specific miRNA alterations identified by NGS under Specific Aim 1 can be used as a 'miRNA classifier' to predict the degree of neuroendocrine differentiation in prostate cancer.

**What was accomplished under these goals?**

In the previous grant year, we made significant progress in understanding the mechanistic role of miRNA genes that formed a part of 'miRNA classifier' that we deduced to be associated with progression of advanced CRPC with adenocarcinoma characteristics to CRPC-NE states as detailed below:

**Specific Aim 1: Examine the dysregulated microRNA repertoire of neuroendocrine prostate cancer**

**Major Task 1: Perform small RNA sequencing in cells from 'in vitro' induced models of neuroendocrine prostate cancer**

Accomplished in Year 1

**Major Task 2: Perform small RNA sequencing in microdissected PCa clinical tissues from CRPC-Adeno vs CRPC-NE** (CRPC-Adeno, n=15; CRPC-NE, n=15)

Accomplished in Year 2

We demonstrated that progression of advanced CRPC with adenocarcinoma characteristics (CRPC-Adeno) to therapy-induced, androgen-independent NE (CRPC-NE) states is associated with a characteristic set of miRNA alterations that promote plasticity of advanced prostate adenocarcinomas to NEPC (Bhagirath et al., Oncogene, 2020). By next generation sequencing (NGS) analyses on Illumina NextSeq platform, we probed the miRNA repertoire of CRPC-Adeno vs CRPC-NE clinical samples and identified a set of dysregulated miRNAs in two independent cohorts (discovery and validation cohort 1, CRPC-Adeno, n=39 and CRPC-NE, n=8). Importantly, we could develop a 'novel miRNA classifier' to robustly stratify CRPC-NE tumors from CRPC-Adenocarcinomas by applying machine learning algorithm (random forest machine learning technique with leave-pair-out cross validation (LPOCV)). We further validated the classifier in two additional independent clinical cohorts (validation cohort 2 and validation cohort 3) of mCRPC samples (total n=24; n=12 each of CRPC-Adeno and CRPC-NE). The two cohorts were acquired from two independent sites (Institute of Cancer Research, UK and Prostate Cancer Biorepository Network, University of Washington site. The performance of classifier was further measured using ROC analyses that showed AUC =0.8318. We further deduced the optimal miRNA genes required for NEPC diagnosis. We applied the '43-miRNA classifier data' we deduced earlier to two independent validation cohorts. This modeling validated a set of 5 miRNAs of the classifier to be important in distinguishing between CRPC-Adeno vs CRPC-NE. Importantly, modeling in these cohorts preserved the following features of the classifier: miRs-10a-5p, -28-3p, -92b-3p, -375 and miR-181b-5p. In the last grant year, we focused on validating the 5-miRNA classifier and determining the functional significance of miRNA alterations.

*Milestone(s) Achieved: Identification and validation of significant miRNA alterations associated with NE prostate cancer.*

**Specific Aim 2: Determine the functional role of top dysregulated miRNAs in NED in prostate cancer**

**Major Task 1: *In vitro* studies to establish the role of the proposed miRNA genes in neuroendocrine prostate cancer**

Included in the main goals of the proposal is to determine the functional significance of dysregulated miRNA genes in NEPC. In keeping with this goal, we focused on deciphering the functional role of top dysregulated miRNAs that were found to be a part of our miRNA classifier. In addition, we had originally proposed to study miR-410. Please note that in lieu of miR-17~92 cluster, an alternate proposed miR was miR-363. In last year's progress report, we included data on miR-410 and miR-106a~363 cluster. In the last grant year, we continued our studies on miR-410. Furthermore, since downregulation of miR-28-3p constituted one of the top features of our '5-miRNA classifier', we focused on elucidating the functional role of this miRNA in NEPC.

**Subtask 1: Generate stable cell lines for miRNA overexpression/knockdown**

Accomplished in Year 1 and 2. In addition, we generated stable cell lines overexpressing miR-28-3p.

**Subtask 2: Perform *in vitro* functional assays with stable cell lines**

Accomplished in Year 1 and 2.

For miR-28-3p, we performed following functional assays with stable cell lines:

- Cell viability/proliferation assays
- Cell cycle and apoptosis assays
- *In vitro* invasion and migration assays
- Assessment of neuronal markers by Western blotting and real time PCR

**Subtask 3: Identify microRNA targets by Western blotting and real time PCR. Validate miRNA targets by constructing luciferase reporter constructs and transiently transfecting into cell lines**

Western blotting and real time PCR for target genes were performed for miR-410 and miR-28-3p. These studies will be continued in the next period.

*Milestone(s) Achieved: Production of stable cell lines capable of inducing miRNA overexpression. Assessment of in vitro effects of proposed miRs in PCa cell lines and identification of miR targets.*

**Major Task 2**: *In vivo* studies to establish the role of the proposed miRNA genes in neuroendocrine prostate cancer

**Subtask 1:** Submit documents for IACUC approval

**Milestone(s) Achieved:** Obtain IACUC approval

**Subtask 2:** Implantation of stable cell lines produced in task 2.1 for miR-410, and monitoring of tumors in mice

As proposed, we generated PCa cell lines modulated for miR-410 expression followed by *in vivo* studies. We generated stable cell lines for overexpression of miR-410 in PC3 and C42B cells. As a control, a scrambled miRNA control was stably transfected into these cell lines. These cells were implanted subcutaneously into nude mice and tumor progression was monitored at regular intervals.

**Subtask 3:** Implantation of stable cell lines produced in task 2.1 for second miRNA gene and monitoring of tumors in mice

We implanted stable cell lines expressing control/miR-28-3p in nude mice followed by monitoring of tumor growth.

**Subtask 4:** Characterization of tumors to analyze miRNA targets

We harvested tumors from miR-410 experiment followed by analyses of miR-410 in harvested tumors. We also analyzed the expression of neuronal genes in these tumors by real time PCR. miRNA targets for miR-410 are being analyzed. Analyses of miR-28-3p targets is ongoing and will be completed in the next year.

*Milestone(s) Achieved: Characterization of effects of proposed miRs on tumor growth in vivo.*

**RESULTS:**

**miR-410 plays an oncogenic role *in vivo***

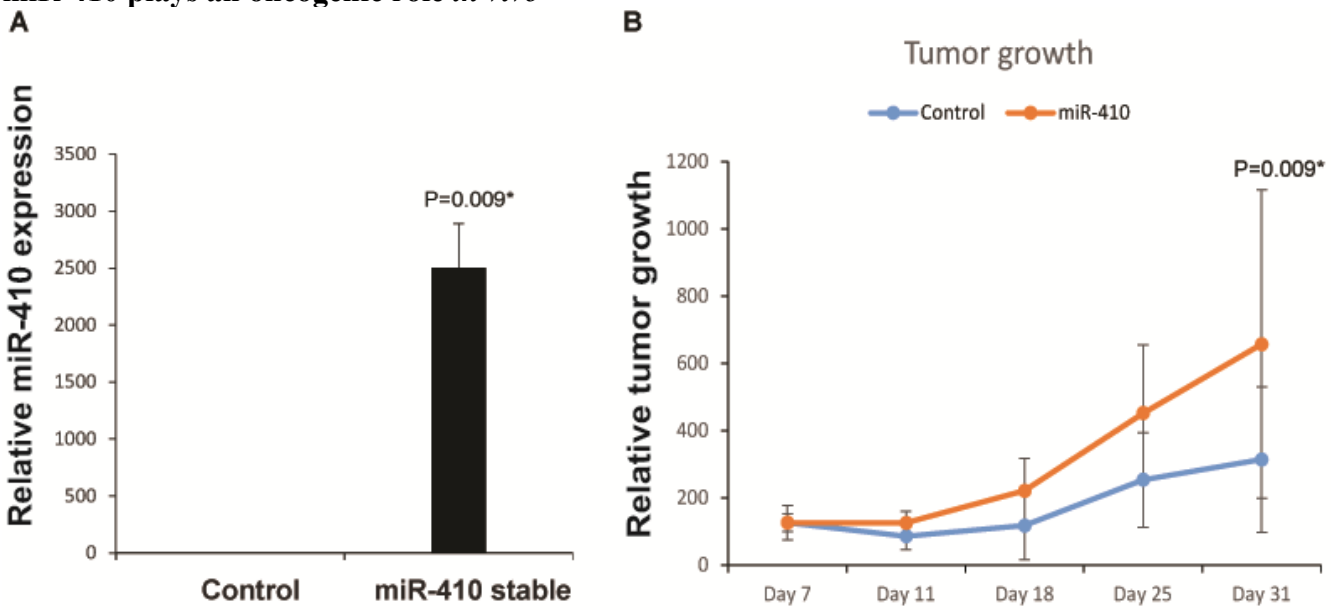


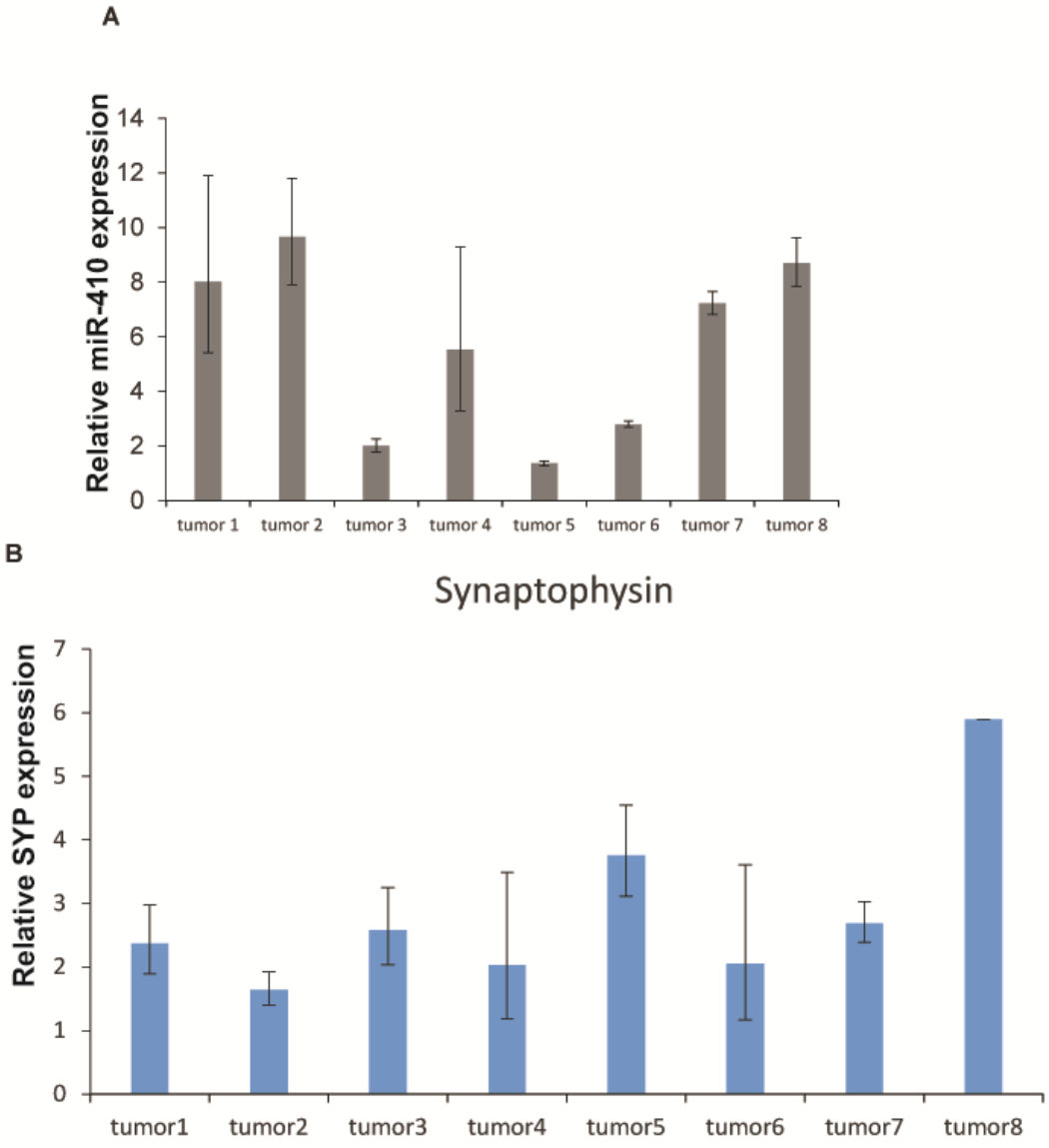

**Fig. 1 miR-410 plays an oncogenic role *in vivo.* A.** Real time PCR based analyses of stable miR-410 transfectants. B. *In vivo* tumor growth of control/miR-410 expressing PC3 cells at indicated time points.

Fig. 1A shows the real time PCR based analyses of stable miR-410 transfectants, As a control, a scrambled miRNA control was stably transfected into PC3 cells. Control/miR-410 overxpressing cells were implanted subcutaneously into nude mice. Immunodeficient mice, nu/nu (Charles River Laboratories) were used for generating xenograft models. Control or test miR overexpressing PC3 cells, each at $3\times10^6$, were resuspended in 50 µL of serum-free culture media and an equal volume of Matrigel (BD Bioscience, MD) and subsequently transplanted s.c. by a syringe fitted with a 27-gauge needle into the right flanks of mice. The mice body weight and tumor sizes were measured at different time points for 31 days. These studies showed that miR-410 overexpressing tumors show an augmented growth, consistent with oncogenic role of this miRNA (Fig. 1B).

**miR-410 regulates the expression of neuronal markers** We further analyzed the tumors from *in vivo* study (Fig. 1B) in control/miR-410 transfected xenografts. Tumors were harvested and RNA was extracted from harvested tumors followed by RT-PCR analyses to assess the expression of miR-410 (Fig. 2A) and NE markers (Fig. 2B). Real time PCR analyses of miR-410 expression confirmed the overexpression of miR-410 in harvested tumors (Fig 2A). Analyses of neuronal markers showed that expression of Synaptophysin was significantly increased in miR-410 expressing tumors as compared to corresponding controls (Fig. 2B). These data suggest that miR-410 regulates the expression of this neuronal marker.

**miR-410 targets:** To identify miR-410 targets, we performed *in vitro* studies. As detailed in last report, we had performed microarray analyses of miR-410 expressing PCa cell lines to examine potential targets of miR-410. Pathway analyses of dysregulated genes in PC3 cells showed a predominant dysregulation of PI3K-Akt-mTOR signaling, epithelila-to-mesenchymal transition (EMT), focal adhesion and regulation of actin cytoskeleton. In the last grant period, we examined the potential direct regulation of these genes by miR-410. However, we could not validate a potential miR-410 target yet. In the next period, we plan to extend these studies to understand the mechanistic basis of observed miR-410 effects in prostate cancer.

**Fig. 2 miR-410 regulates the expression of Synaptophysin A.** Real time PCR based analyses of stable miR-410 transfectants. B. *In vivo* tumor growth of control/miR-410 expressing PC3 cells at indicated time points.



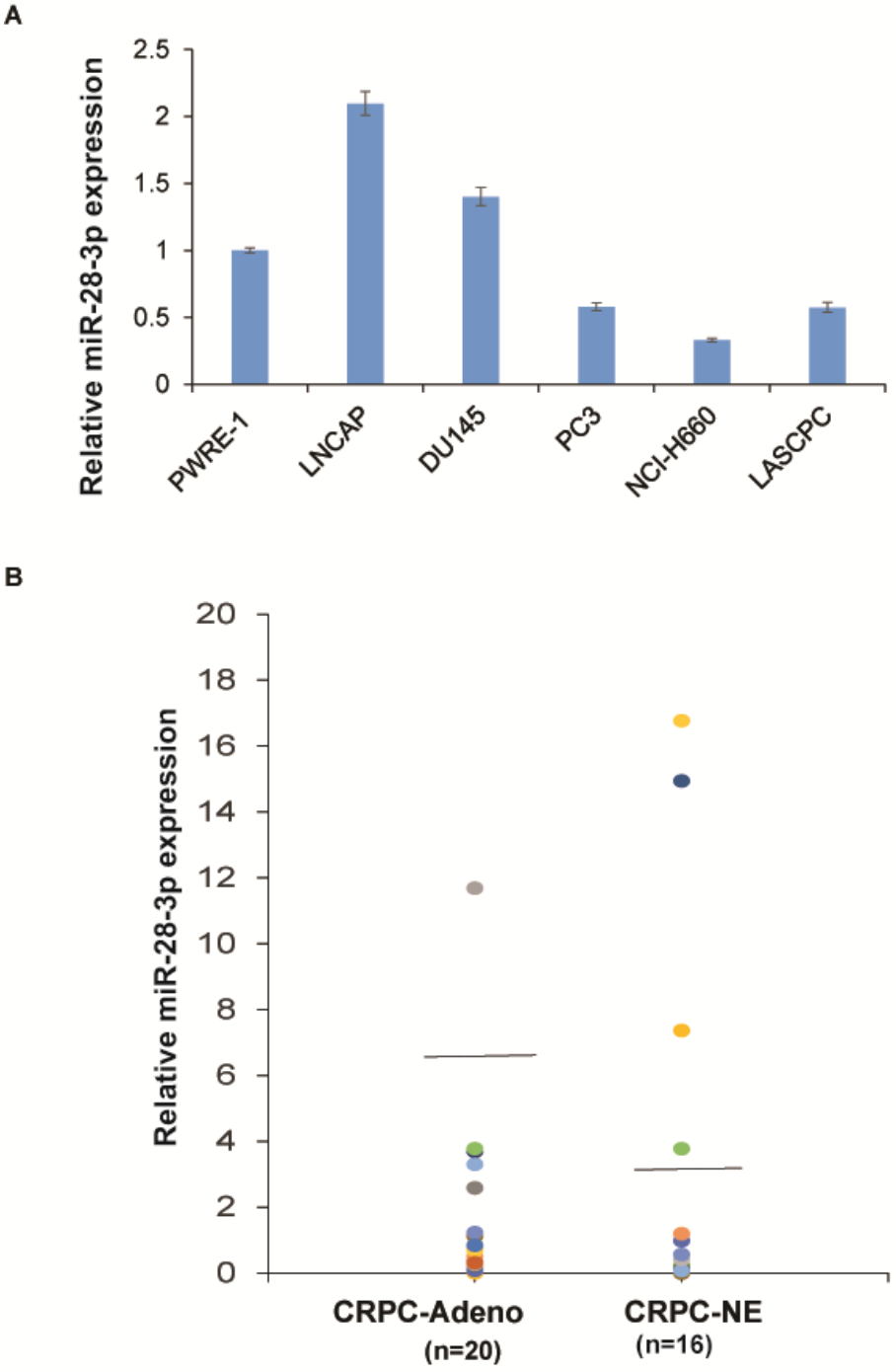

Since downregulation of miR-28-3p constituted one of the top features of our '5-miRNA classifier', we focused on elucidating the functional role of miR-28-3p in NEPC as detailed below:

**miR-28-3p is downregulated in neuroendocrine prostate cancer**

We examined the expression of miR-28-3p in prostate cancer cell lines (Fig. 3A). Our analyses showed that as compared to normal immortalized prostate epithelial cell line PWR1E, prostate cancer cell lines LNCaP and Du145 and C42B had a higher expression of miR-28-3p. However, PC3 cells and NEPC cell lines, NCI-H660 and LASCPC-01 were found to have lower expression of miR-410-3p as compared to PWR1E cells. These data suggest that this miRNA potentially has a biphasic role in prostate cancer with its expression increased in primary prostate cancer and decreasing expression with advanced disease. We further analyzed miR-28-3p expression in microdissected CRPC-Adeno and CRPC-NE tissues by real time PCR (Fig. 3B). Our analyses showed that the average expression of miR-28-3p is lower in CRCP-NE tissues as compared to CRPC-

adenocarcinomas. NGS analyses of CRPC-Adeno vs CRPC-NE samples done under Specific Aim 1 also showed decreased expression of this miRNA in NEPC.

**Fig. 3 miR-28-3p is downregulated in neuroendocrine prostate cancer**
A. Real time PCR based expression analyses of miR-28-3p expression in normal immortalized prostate cell line PWRE-1, primary prostate cancer cell lines and neuroendocrine prostate cancer cell lines NCI-H660 and LASCPC01. B. Real time PCR analyses of miR-410 expression in microdissected CRPC-Adeno vs CRPC-NE clinical tissues.

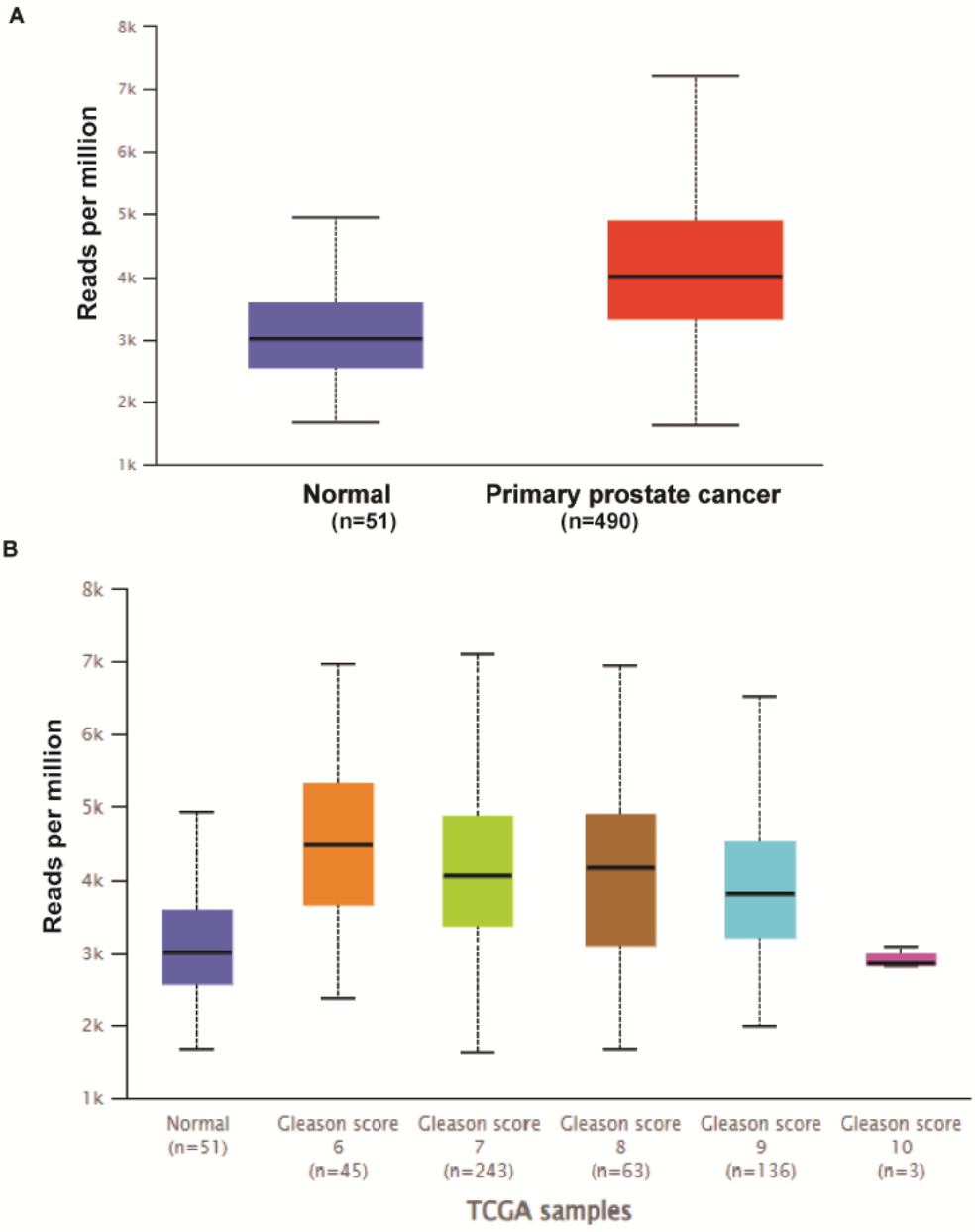


**A potential biphasic role of miR-28-3p in prostate cancer**

In view of these expression data in clinical tissues and cell lines, we extended miR-28-3p expression analyses in primary prostate cancer (Fig. 4). We probed the primary prostate adenocarcinomas in the Cancer Genome Atlas dataset for miR-28-3p expression using UALCAN portal [1] (Fig. 4A ). Our analyses showed that as compared to normal tissues (n=51), primary prostate adenocarcinomas (n=490) show a significantly upregulated expression of this miRNA (P=0.001*). In view of this data and our data pointing to a potential biphasic role of this miRNA, we further examined the pattern of miR-28-3p expression across various Gleason scores of the disease (Fig. 4B). Interestingly, while miR-28-3p is highly upregulated in Gleason score 6 primary prostate tumors, with increasing Gleason score, the expression of this miRNA decreases. These findings are in line with our hypothesis.

**Fig. 4 A potential biphasic role of miR-28-3p in prostate cancer**

A. miR-410 expression in TCGA dataset of primary prostate adenocarcinomas (n=490) vs normal (n=51). B. miR-410 expression analyses in TCGA dataset of prostate adenocarcinomas stratified by Gleason score.

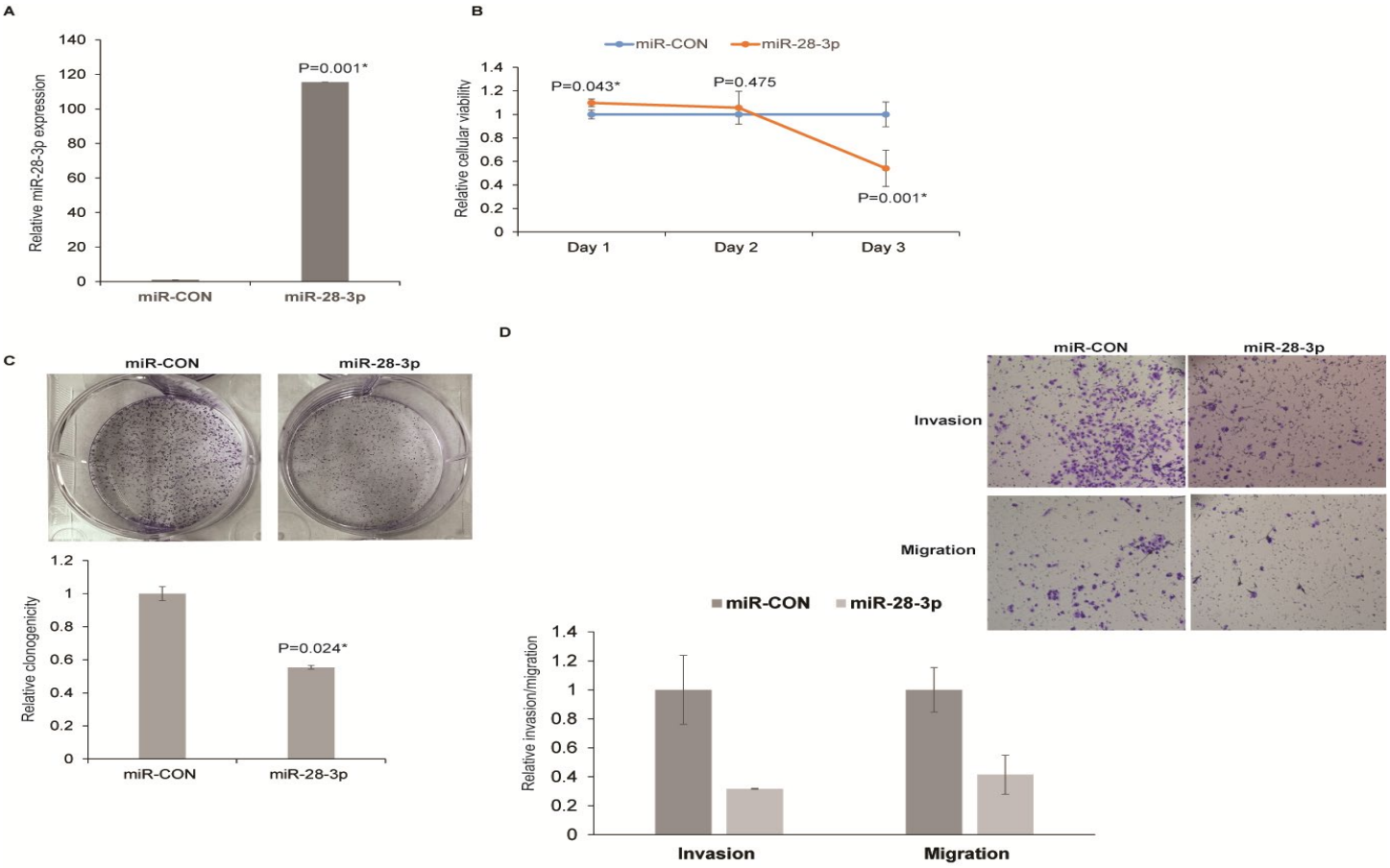


**miR-28-3p is a tumor suppressive miRNA in advanced prostate cancer**

We further sought to examine the functional role of this miRNA in advanced prostate cancer. Towards this, we overexpressed control miRNA/miR-28-3p in PC3 cells followed by functional assays. miR-28-3p was overexpressed in PC3 cells by transient transfection of miR-28-3p mimic (Life Technologies) for 72 hours. As a control, miR-CON (Life Technologies) was transfected in PC3 cells. Real time PCR confirmed overexpression of miR-28-3p in transfected cells as compared to control (Fig. 5A). Analyses of cellular viability by MTS assay (Fig. 5B) showed that cellular viability significantly decreases on Day 3 in PC3 cells transfected with miR-28-3p as compared to control. Clonogenicity assay confirmed that miR-28-3p transfectants have lower proliferative ability as compared to miR-CON transfected cells (Fig. 5C). To examine the role of miR-28-3p in influencing *in vitro* invasiveness and migratory ability of PCa cell lines, transwell invasion and migration assays were conducted (Fig. 5D). miR-28-3p overexpression led to decreased migratory and invasive abilities in PC3 cells as compared to control. These data point to a tumor suppressive role of miR-28-3p in PC3 cells.

**Fig. 5 miR-28-3p is a tumor suppressive miRNA in advanced prostate cancer**

A. Real time PCR based assessment of miR-28-3p levels in PC3 cells transfected with miR-CON/miR-28-3p mimics for 72 hours B. MTS cellular viability assay; C. clonogenicity assay and D. *In vitro* migration and invasion assay in miR-CON vs miR-28-3p transfected PC3 cells.



**miR-28-3p directly represses mesenchymal gene Vimentin and oncogenic AKT3 in prostate cancer cells**
We next examined potential target genes for miR-28-3p by Western blot analyses. *In silico* analyses with Targetscan showed that Vimentin and AKT3 are potential target genes for miR-28-3p as these genes have a potential miR-28-3p binding site within their respective 3' untranslated regions (UTRs) (Fig. 6A). Western blot analyses showed that miR-28-3p overexpression causes decreased expression of Vimentin and AKT3 (Fig. 6B), validating these genes as direct miR-28-3p targets. These data suggest that loss of miR-28-3p expression in late stage prostate cancer leads to induction of mesenchymal Vimentin, leading to induction of epithelial-to-mesenchymal transition (EMT) and metastasis. Since EMT plays an important role in driving neuroendocrine differentiation in prostate cancer, we propose that this miRNA regulates NED via its regulation of EMT. In the next period, we will validate this hypothesis.

**Fig. 6 miR-28-3p directly represses mesenchymal gene Vimentin and oncogenic AKT3 in prostate cancer cells** A. Schematic representation of 3' UTR of VImentin and AKT3 showing putative miR-28-3p binding sites in their 3' UTR regions. C. Western blot analyses in miR-CON/miR-28-3p transfected PC3 cells


**Specific Aim 3**: **Determine the prognostic potential of miRNAs regulating NEPC**

**Subtask 1: Collection of retrospective tissue samples (CRPC-Adeno, n=30; CRPC-NE, n=20)**

We originally proposed following tissue numbers in proposal:

Specific Aim 1: CRPC-Adeno, n=15; CRPC-NE, n=15: To be analyzed by small RNA-next generation sequencing

Specific Aim 3: CRPC-Adeno, n=30; CRPC-NE, n=20: To be analyzed by real time PCR.

As proposed, we have conducted NGS analyses under Specific Aim 1. However, to ensure the robustness of our classifier, we have analyzed additional samples proposed under Specific Aim 3 (CRPC-Adeno, n=47; CRPC-NE, n=15) by NGS analyses as well.

Considering all the samples sequenced so far, we have completed our analyses for following total numbers:

CRPC-Adeno, n=56 (n=15 under Aim 1 and n=47 under Aim 3); CRPC-NE: n=30 (n=15 under Aim 1 and n=15 under Aim 3). This includes analyses of CRPC-Adeno and CRPC-NE samples from two independent sites (Institute of Cancer Research, UK) and Prostate Cancer Biorepository Network, University of Washington site. In the last grant year, retrospective CRPC-Adeno (n= 11) and CRPC-NE samples (n=8) were collected from Augusta University. CRPC-adeno included metastatic CRPC patients with no evidence of NED while CRPC-NE included metastatic AR- patients with therapy-induced NED with features of small cell/large cell NE carcinoma.

**Subtask 2: Microdissection of tissue samples and extraction of RNA**

FFPE tissues slides were H&E stained, marked by Dr Patel for tumor areas and microdissected. RNA were isolated from microdissected tissues by employing miRNeasy FFPE kit (Qiagen) as per manufacturer's instructions and quality of RNA was examined on Agilent Bioanalyzer.

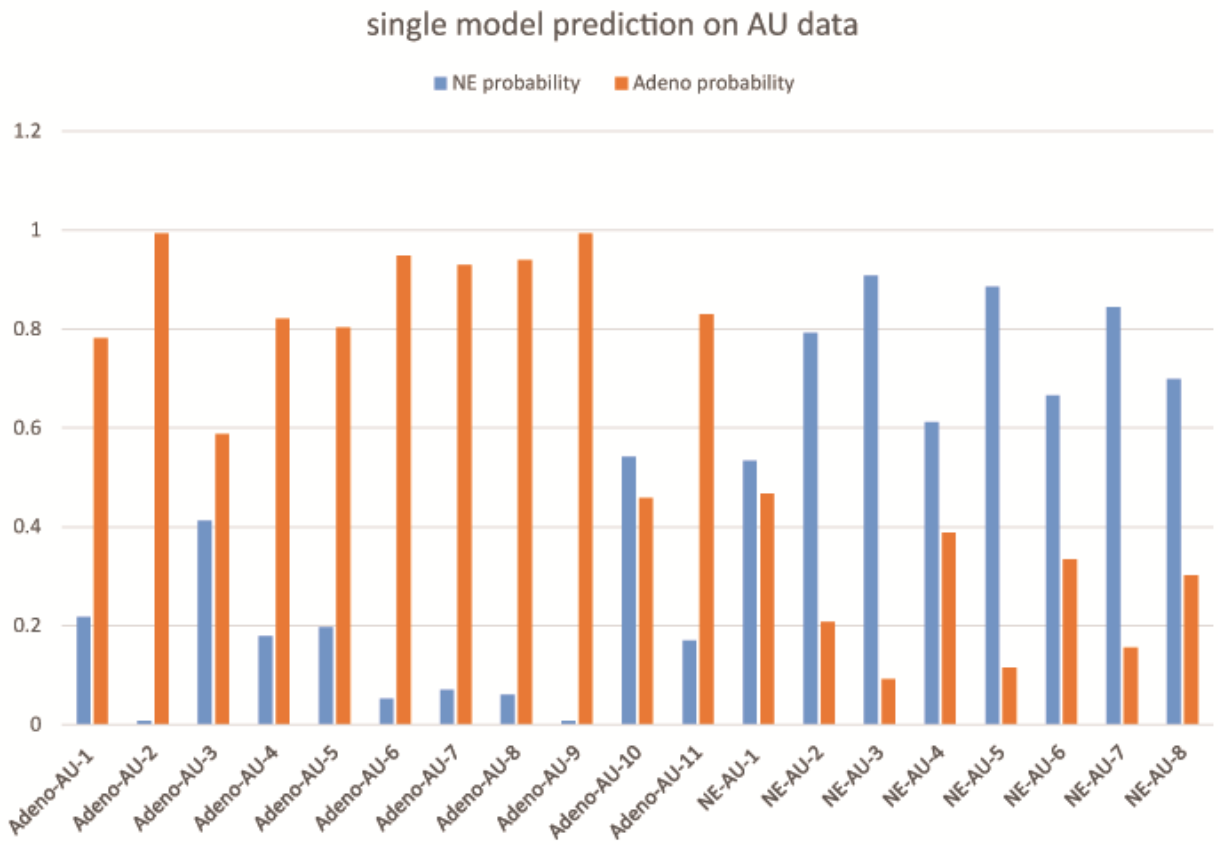
**Subtask 3: Profiling of significant miRNAs in clinical samples and PDX models**

For clinical samples, small RNA sequencing was performed at Augusta University molecular core and analyzed by Dr. Sharma's group. The validated 'miRNA classifier' was tested in LuCaP models by real time PCR.

**RESULTS**

1. **Analyses of clinical samples:** A retrospective cohort of CRPC-adeno vs CRPC-NE samples were analyzed by small RNA Next Generation Sequencing Using 0.5µg of purified total RNA, libraries were generated using an Illumina® TruSeq® small RNA library prep kit (cat no. RS-200-0012) as per manufacturer's instructions. Index libraries were equally pooled and sequenced on Illumina NextSeq 500 platform at the Augusta University molecular core facility using an Illumina NextSeq® 500/550 Mid Output Kit v2 (150 cycles). Sequencing reads were adapter trimmed and analyzed by BaseSpace Small RNA app (Illumina) and Dr. Sharma's group. We further examined if the set of miRNAs included in our 'miRNA classifier' we deduced earlier (Bhagirath et al., Oncogene, 2020) could distinguish/classify NEs from adenocarcinomas in this cohort. Towards this, we applied the 'classifier data' obtained from our discovery cohort 1 (Bhagirath et al., Oncogene, 2020) to AU validation cohort by employing single model prediction algorithm (Fig. 7). Fig. 7 shows the prediction probabilities of a clinical sample to be 'adenocarcinomas' (blue) or 'NE' (orange) based on our 'miRNA classifier'. Interestingly, miRNA classifier could correctly stratify 10/11 adenocarcinomas and 7/8 NE samples. The prediction probabilities for adenocarcinomas ranged from 0.58 to 0.99 with concomitant low NE probabilities while the classifier correctly predicted high NE probabilities of the included CRPC-NE cases with concomitant low adeno probabilities. These data validate the robustness of our miRNA classifier to distinguish between 'Adeno' vs 'NE' states. The performance of classifier was further measured using ROC analyses that showed AUC =0.95. These data strongly support the robustness of our 'miRNA classifier'.
2. **Real time PCR based validation of miRNA classifier in patient-derived xenograft (PDX) models:** RNA samples for PDX models with with adenocarcinoma histology (LuCaP 70, 78, 81) vs NE histology (LuCAP 49, 145.1, 145.2) were procured from Prostate Cancer Biosrepository Network. We validated the 5 miRNA classifier (miRs-10a-5p, -28-3p, -92b-3p, -375 and miR-181b-5p) in these samples by real time PCR using Taqman MicroRNA assays (Life Technologies). RNU48 was used as an endogenous control. Real time PCR based profiling showed that miR-375 (Fig. 8A), miR-92b-3p (Fig. 8D) and miR-10a-5p (Fig. 8E) were upregulated in CRPC-NE PDX models while miR-28-3p (Fig. 8C) and miR-181b-5p (Fig. 8B) were downregulated as compared to CRPC-Adeno models. Please note that the shown graphs show relative dCt values.

**Fig. 7 MicroRNA classifier is robust in distinguishing between CRPC-Adeno and CRPC-NE cases.** Prediction probabilities for samples in AU cohort to be 'adenocarcinoma' (orange) or 'NE' (blue) histology based on miRNA classifier.

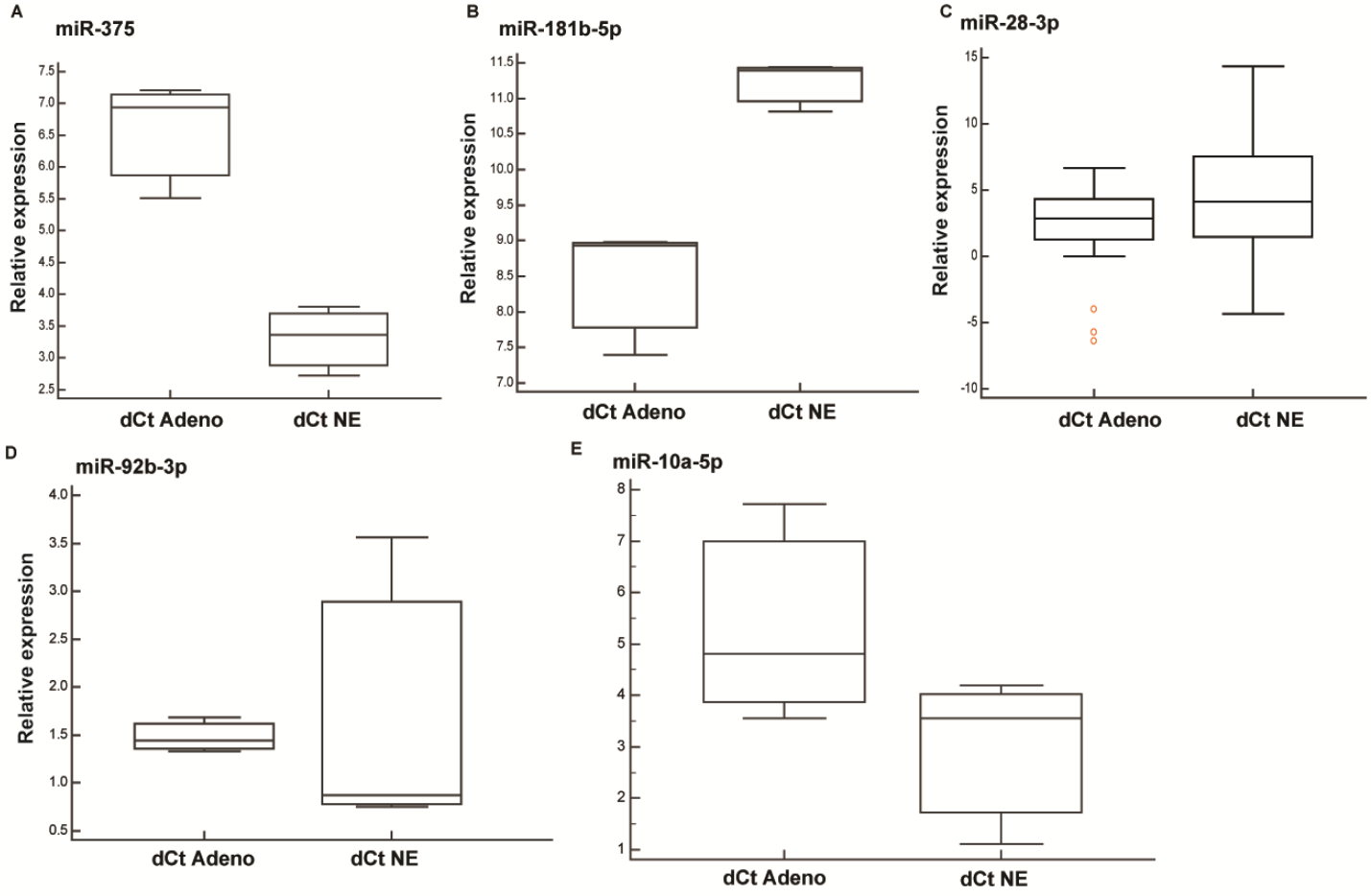



**Subtask 4: Statistical analyses to correlate miRNA expression with clinical data**

These analyses will be performed in the next year.

*Milestone(s) Achieved: Validation of significantly dysregulated 'NE-specific' miRNAs, identification of predictive/prognostic NED indicators.*

**Fig. 8 Real time PCR based validation of miRNA classifier in patient-derived xenograft (PDX) models.** RNA samples for PDX models with adenocarcinoma histology (LuCaP 70, 78, 81) vs NE histology (LuCAP 49, 145.1, 145.2) were employed for real time PCR based analyses of indicated miRNAs. RNU48 was used as an endogenous control.




## REFERENCES


1 Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B *et al*. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia 2017; 19: 649-658.


## What opportunities for training and professional development has the project provided?

Nothing to report

## How were the results disseminated to communities of interest?

Results were disseminated at the following conference presentations:

**POSTER PRESENTATIONS**:

- Saini S*, Bhagirath D, Patel N, Sharma A, Lee TJ and Saini S*. A microRNA-based classifier for identification of neuroendocrine differentiation in metastatic castration-resistant prostate cancer. ASCO Genitourinary Cancers Symposium, 2022
- Saini S*, Bhagirath D, Patel N, Sharma A, Lee TJ and Saini S*. A microRNA-based classifier for identification of neuroendocrine differentiation in metastatic castration-resistant prostate cancer. Annual Meeting of American Association of Cancer Research, 2022
- Sreekumar A, Patel N and Saini S*. Regulatory role of miR-410 in prostate cancer. Annual Meeting of American Association of Cancer Research, 2022.

**ORAL PRESENTATION:**

- Saini S. Molecular determinants of neuroendocrine prostate cancer. Invited presentation at Amity University, India. June 2022

**What do you plan to do during the next reporting period to accomplish the goals?**

- **Determine the predictive/prognostic potential of 'microRNA classifier':** We will continue our studies on validation of our deduced miRNA classifier. We will correlate the miRNA classifier with clinicopathological parameters of the disease to determine its prognostic/predictive potential. Validation of the classifier will have profound translational implications as it will provide a novel way to classify and predict NED in CRPC patients.

- ***In vitro* functional studies on proposed miRNA miR-410 and miR-28-3p to determine their roles in NEPC.** In order to gain a comprehensive understanding of the role of miR-410 and miR-28-3p in prostate cancer, we will continue our studies during the next year. We will identify additional miRNA targets by Western blotting and real time PCR, validate miRNA targets by luciferase reporter assays and functional assays.

- ***In vivo* studies on proposed miRNAs to establish the role of the proposed miRNA genes in neuroendocrine prostate cancer.** We will generate xenograft tumors with PCa cell lines modulated for miR-28-3p levels followed by monitoring of tumor growth. We will harvest the tumors and examine the target genes by Western blotting and immunohistochemistry.

## 4. IMPACT: What was the impact on the development of the principal discipline(s) of the project?

- Our microRNA-based classifier has potential translational implications. It has the potential to transform the diagnosis of neuroendocrine prostate cancer that is currently challenging. Currently, there are limited molecular biomarkers for diagnosing aggressive neuroendocrine prostate cancer that makes it challenging to treat this variant with the right drugs.
- Our studies have identified novel miRNA mediated signaling pathways instrumental in driving neuroendocrine differentiation in advanced prostate cancer. These findings will increase the understanding of the mechanistic basis of the disease with potential of translation into novel therapeutic approaches for treatment of NEPC.

**What was the impact on other disciplines?**

Nothing to report

**What was the impact on technology transfer?**

Nothing to report

**What was the impact on society beyond science and technology?**

Nothing to report

## 5. Changes/Problems

Nothing to report

**Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report

**• Changes that had a significant impact on expenditures**

The project was on hold from July 1, 2020 through June 14, 2021 as PI was on medical leave. Therefore, expenditures on personnel and supplies were on hold during this period. The project resumed in June 2021 and the carryover is being used for current personnel working under the project.

**• Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select Agents**

Nothing to report

**• Significant changes in use or care of human subjects**

Nothing to report

• **Significant changes in use or care of vertebrate animals.**

Nothing to report

• **Significant changes in use of biohazards and/or select agents**

Nothing to report

## 6. Products

**Publications, conference papers, and presentations**

Sreekumar A and Saini S. Role of transcription factors and chromatin modifiers in driving lineage reprogramming in treatment- induced neuroendocrine prostate cancer. Frontiers in Cell and Developmental Biology (under review).

Bhagirath D, Liston M, Lui B, Yang TL, Olshen A, Tabatabai LZ, Dahiya R, Majid S and Saini S. A microRNA signature of neuroendocrine differentiation in metastatic castration-resistant prostate cancer. Oncogene. 2020 Oct 9. doi: 10.1038/s41388-020-01493-8. Online ahead of print. PMID: 33037409. Acknowledgement of federal support: Yes

Bhagirath D, Liston M, Akoto T, Lui B, Bensing B, Sharma A and Saini S. Novel, non-invasive markers for detecting therapy induced neuroendocrine differentiation in castration-resistant prostate cancer patients. Scientific Reports. Sci Rep. 2021; 11: 8279. Published online 2021 Apr 15. doi: 10.1038/s41598-021-87441-2. PMCID: PMC8050049. Acknowledgement of federal support: Yes

Akoto T, Bhagirath D and Saini S. MicroRNAs in treatment-induced neuroendocrine differentiation in prostate cancer. Review article for Special Issue-"Resistance in Endocrine Therapy of Prostate Cancer" for journal:Cancer Drug Resist 2020;3:[Online First].10.20517/cdr.2020.30.

Bhagirath D, Dahiya R, Majid S, Tabatabai ZL and Saini S. Sequencing small non-coding RNA from formalin-fixed tissues and serum-derived exosomes from castration-resistant prostate cancer patients. J Vis Exp. 2019 Nov 19;(153). doi: 10.3791/60549.Acknowledgement of federal support: Yes

Bhagirath D, Yang YL, Tabatabai ZL, Majid S, Dahiya R, Tanaka Y and Saini S. BRN4 is a novel driver of neuroendocrine differentiation in castration-resistant prostate cancer and is selectively released in extracellular vesicles with BRN2. Clin Cancer Res. 2019 Aug 1. pii: clincanres.0498.2019. doi: 10.1158/1078-0432.CCR-19-0498. [Epub ahead of print] PMID: 31371344. Acknowledgement of federal support: Yes

**Other publications, conference papers and presentations**.

**POSTER PRESENTATIONS**:

- Saini S*, Bhagirath D, Patel N, Sharma A, Lee TJ and Saini S*. A microRNA-based classifier for identification of neuroendocrine differentiation in metastatic castration-resistant prostate cancer. ASCO Genitourinary Cancers Symposium, 2022
- Saini S*, Bhagirath D, Patel N, Sharma A, Lee TJ and Saini S*. A microRNA-based classifier for identification of neuroendocrine differentiation in metastatic castration-resistant prostate cancer. Annual Meeting of American Association of Cancer Research, 2022
- Sreekumar A, Patel N and Saini S*. Regulatory role of miR-410 in prostate cancer. Annual Meeting of American Association of Cancer Research, 2022.

**ORAL PRESENTATION:**

- Saini S. Molecular determinants of neuroendocrine prostate cancer. Invited presentation at Amity University, India. June 2022

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

## 7. Participants & Other Collaborating Organizations

Sharanjot Saini, PI: No change

**Coinvestigators:**
**Name:** Dr. Ashok Sharma
**Project Role:** Bioinformatician
**Nearest person month worked**: 0.6
**Contribution to Project:** Dr. Ashok Sharma is a bioinformatician at Augusta University who helped in analyses of NGS data and validation of the miRNA classifier.

**Name:** Dr. Nikhil Patel
**Project Role:** Pathologist
**Nearest person month worked**: 0.6
**Contribution to Project:** Dr. Patel is a pathologist at Augusta University who helped in characterization of clinical tissues used in the project.

**Other personnel:**

**Name:** Amritha Sreekumar
**Project Role:** Research Associate
**Nearest person month worked**: 12
**Contribution to Project:** Amritha Sreekumar carried out mechanistic studies on deciphering the role of important microRNAs in neuroendocrine prostate cancer.

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

**What other organizations were involved as partners?**

Nothing to report

## 8. Special Reporting Requirements

## 9. Appendices: None