**miRNA 26a Expression in a Novel Panel of African American Prostate Cancer Cell Lines**

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**INTRODUCTION**

African American men have disproportionately high incidence and mortality rates of prostate cancer when compared to other ethnic groups in the United States. The identification of molecular factors that contribute to this disparity could improve diagnosis and therapeutic intervention. Therefore, the purpose of this study was to determine the miRNA 26a expression profile in novel African American and Caucasian prostate cell lines at each clinical stage of prostate cancer progression.

**Methods:** The miR-26a expression profile was investigated using novel African American and Caucasian prostate cell lines representing each pathological stage: non-malignant, malignant, and metastatic tumors. Relative miRNA expression was determined by qRT-PCR.

**Results:** Our data showed a 2.25 fold increase for miR-26a in the non-malignant, a 13.3 fold increase in malignant and 2.38 fold increase in metastatic tumors, when comparing African American and Caucasian prostate cell lines of similar clinical stage and pathological grade. African American malignant prostate cancer cell lines showed the most significant fold difference in expression among all cell lines tested. Furthermore, there was a general increase in miR-26a expression toward the more aggressive cell lines in both African American and Caucasian prostate cell lines.

**Conclusion:** To date, we are unaware of any studies that compare the miRNA profile at different stages of prostate cancer among two racial groups. Although a gene target for miR-26a has not been identified, our data show a possible role for miRNA regulation of gene expression in prostate cancer progression. Furthermore, this study suggests that miRNAs could possibly contribute to the aggressiveness associated with African American prostate cancers.

**Key Words:** miRNA, African American Prostate Cancer

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**MATERIALS AND METHODS**

To determine the expression pattern of miR-26a, we assembled a panel of

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Table 1. Clinical features of African American and Caucasian patients of whom prostate cell lines were derived

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Age</th>
<th>Race</th>
<th>Morphology</th>
<th>Clinical Stage</th>
<th>Tumor Grade</th>
<th>Gleason Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC77N</td>
<td>62</td>
<td>AA</td>
<td>Epithelial</td>
<td>Non-malignant</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>RC77T</td>
<td>62</td>
<td>AA</td>
<td>Epithelial</td>
<td>Primary adenocarcinoma</td>
<td>Poorly differentiated</td>
<td>7</td>
</tr>
<tr>
<td>MDA-2Pca-2b</td>
<td>63</td>
<td>AA</td>
<td>Epithelial</td>
<td>Adenocarcinoma</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>PrEC</td>
<td>59</td>
<td>White</td>
<td>Epithelial</td>
<td>Non-malignant</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>RC-92a</td>
<td>57</td>
<td>White</td>
<td>Epithelial</td>
<td>Primary adenocarcinoma</td>
<td>Well-differentiated</td>
<td>3 + 3</td>
</tr>
<tr>
<td>PC-3</td>
<td>62</td>
<td>White</td>
<td>Epithelial</td>
<td>Metastatic adenocarcinoma</td>
<td>Undifferentiated</td>
<td>NA</td>
</tr>
</tbody>
</table>

AA = African American.
NA = not applicable.
NA' = Not available.

Table represents the age, ethnicity, morphology, clinical and pathological features of the patients from whom these cell lines were obtained.

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miRNA 26a is highly conserved across species and linked to chromosome 7. Real-time quantitative (qRT-PCR) expression of hsa-miR-26a was completed using the miRNA specific Taqman miRNA assay primer sets, reagents and probes (Applied Biosystems, Foster City, Calif.). qRT-PCR were performed according to manufacturer protocols. RNU6B was used to normalize all RNA samples. RNA concentrations were determined with a NanoDrop apparatus (NanoDropTechnologies, Inc., Wilmington, Del.) and 10 nanogram per sample was used for the assays. RNA was reverse transcribed using the primer sequence UUCAAGUAAUCCAG-GAUAGGC at +16°C for 30 min, +42°C for 30 min, and +85°C for 5 min at which enzyme inactivation occurs. Step One qRT-PCR (Applied Biosystems) instrument was used to perform amplification of miRNA sequence at +95°C for 10 min for enzyme activation, +95°C for 15 sec, +60°C for 60 sec at 40 cycles. miRNA analysis was performed in triplicates, and fold change was calculated using 2 ^ [-ΔCt] values for hsa-miR-26a in each cell line.

Statistical Analysis
Statistics for all experiments were performed using Microsoft Excel. One way ANOVA was utilized to determine a statistical difference between cell lines at each clinical stage.

RESULTS
Clinical Features of African American and Caucasian Prostate Cell Lines
Of the previously reported non-malignant and malignant African American and Caucasian prostate cell lines, we utilized a novel non-malignant RC-77N and malignant RC-77T prostate cell culture model that originated from an African American patient who underwent radical prostatectomy. The profile of each of these cell lines is shown in (Table 1).

Increased miRNA 26a Expression in African American Cell Lines
miRNA 26a is highly conserved across species and linked to chromo-
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Real time PCR utilizing primer sequences for miR-26a was performed on African American non-malignant RC-77N, malignant RC-77T, and metastatic MDA-PCa-2b and corresponding Caucasian derived cell lines non-malignant PrEC, malignant RC-92a/hTERT and metastatic PC-3 cells. Utilizing Caucasian miR-26a expression as the baseline and a 2-fold threshold difference, we observed significant increases at each stage in the African American derived prostate cells compared to its corresponding Caucasian derived prostate cells (Figure 1 A–C). Specifically, non-malignant RC-77N expressed a 2.25 fold increase in miR-26a expression compared to PrEC cells. This trend continued in the malignant stages with the RC-77T cells expressing a 13.13 fold increase in miR-26a expression compared to RC-92a/hTERT cell. Although not as dramatic as the malignant comparison, we also observed a 2.38 fold increase in metastatic MDA-PCa-2b compared to PC-3 cells (data not shown) (Table 2). Additionally, we were able to observe a general increase in miR-26a expression in our African American prostate model, with RC-77T displaying the most significant expression of all cell lines regardless of stage (Figure 1D).

DISCUSSION

Our understanding of molecular mechanisms associated with increased incidence and mortality in African American prostate cancer has been hampered by the lack of available in vitro model systems to study the complex multistep process of prostate carcinogenesis at various stages of the disease. To date, only two models, E006AA (fresh prostatectomy specimen) and MDA-PCa 2 (from a single metastatic location), are established in vitro models for African American prostate cancer. To our knowledge, this is the first report of a panel of prostate cell lines derived from both African American and Caucasian American males which represent clinically relevant stages of prostate progression (Table 1).

miRNA 26a, a highly conserved miRNA across species, is up-regulated 2.25 fold in non-malignant, 13.13 fold in malignant, and 2.38 fold in metastatic in the African American prostate cancer cell line model compared to the corresponding commonly utilized Caucasian prostate cancer cell line counterpart of a similar stage. (Figure 1). Our data are consistent with the initial report that miR-26a is differentially expressed in non-tumor African American prostate tissue compared to non-tumor Caucasian prostate tissue, and the follow-up report from these same investigators that matched tumors have increased miR-26a as well. Unfortunately, the authors did not indicate whether the matched samples were from African American or Caucasian non-tumor tissues, therefore comparisons to our findings cannot be drawn. While a role for increased miR-26a expression in prostate cancer is supported by several reports, a gene target for miR-26a is not. Therefore, we utilized an indirect in silico method for target gene prediction. Several publicly available prediction software, including PicTar (http://www.pictar.bio.nyu.edu) and TargetScan 3.0. (http://www.targetscan.org/), utilize different algorithms and ranking criteria to generate a partially overlapping set of gene candidates for a given

Table 2. Fold change of hsa-mir-26a by qRT-PCR in African American prostate cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>qRT-PCR fold change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-malignant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RC-77N</td>
<td>2.25</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RC-77T</td>
<td>13.13</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Metastatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA-Pc2b</td>
<td>2.38</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

All fold changes were statistically significant as determined by ANOVA.

The fold change of African American cell lines (n=3) were referenced to Caucasian cell lines (n=3).
miRNA. Interestingly, miR-26a yields a highly diverse spectrum of genes consisting of more than 600 different predicted targets, including genes associated with cell survival and apoptosis. Of the relevant targets, SMAD1, BAK1, and MYC and PTEN gene are known regulators of cell survival and apoptosis.

Since miR-26a increases are associated with more advanced prostate cancer cells (Figure 1A–D), this suggests a role for miR-26a in regulation of apoptosis and cell survival. This role was substantiated in a report that MDA-MB-231 breast cancer cells cultured under hypoxic conditions, show increases in miR-26a expression.18 Thus, the increased incidence associated with African American prostate cancer may be associated with the epigenetic regulation of cell survival or apoptotic genes. This hypothesis is supported by reports that African American prostate cancer patients have altered expression of Bcl-2 (anti apoptosis protein) in more aggressive carcinomas.19 miR-26a expression is increased in our African American non-malignant cell culture model (Table 2) and non-tumor prostate tissue10 suggests a rationale that tumor initiation and subsequent growth at the primary site is increased in African Americans because fewer cells are directed to die. This is substantiated with unpublished data from our laboratory that knock down of miR-26a in DU-145 prostate cancer cells induces caspase 3/7 activation, further highlighting a role for miR-26a in apoptosis resistance. Although it is unresolved why we observe similar fold increases in the non-malignant and metastatic cell lines in our race-specific comparisons, similar expression levels of miR-26a were observed in the RC77T and MDA-PCa2b cell lines, which is consistent with increases in miR-26a in more advanced prostate cancers (Figure 1D). One possible explanation is primary tumor initiation and establishment of metastatic cells are similar processes, where cell survival is a prerequisite to tumor growth,20,21 however, we cannot exclude the possibility of cell line specific differences.

Our analysis of miR-26a in these novel cell lines suggests that this is a relevant model to determine miRNA expression patterns associated with African American prostate cancer. Although identification of gene targets and validation in clinical samples is necessary for implicating miRNA involvement in cancer, these novel cell lines provide an invaluable stage dependent in vitro model to determine race-specific differences associated with African American men with prostate cancer. Although additional studies, involving a large number of patients and the search for miR-26a gene targets, are currently in progress, this panel represents the first prostate cancer cell culture model to study molecular mechanisms associated with the health disparity in African American prostate cancer patients.

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REFERENCES