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

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Abstract

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 in African American patients with prostate cancer.

Keywords

miRNA; African American Prostate Cancer

Introduction

African American men have disproportionately high incidence and mortality rates of prostate cancer when compared to other ethnic groups in the United States, with African Americans having a 19% increased incidence and 37% increased mortality rate compared to

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Caucasian American men.[1] While genetic factors that contribute to higher prostate cancer incidence and mortality in African American men have been suggested, such as increased expression of androgen receptor (AR)[2,3] and epidermal growth factor expression (EGFR),[4] no definitive explanation exists for this disparity. It is reasonable to speculate that epigenetic mechanisms could possibly contribute to the aggressiveness associated with African American prostate cancers.

MicroRNAs (miRNAs) are small, non-coding RNAs that modulate gene expression post-transcriptionally. Hundreds of miRNAs have been identfied, with varying patterns of expression. More than 50% of annotated human miRNA genes are located in fragile chromosomal regions that are susceptible to amplification, deletion, or translocation during the course of tumor development.[5] Moreover, recent evidence indicates that some miRNAs can function either as oncogenes or tumor suppressors,[6,7] and expression profiling analyses have revealed characteristic miRNA signatures in certain human cancers.[6] These finding also reveal that different cancer types share some individual miRNA signatures.[8] Differential expression of miRNAs has been reported in prostate cancer,[9] however the importance of miRNAs in African American prostate cancer has not been fully explored.

The potential involvement of miRNA expression in a race-specific manner was first suggested in the 2006 review by Calin and Croce.[10] Here, they reported that non-tumor African American and Caucasian prostate tissue express divergent miRNA expression patterns, where miRNAs, *mi*R-*301*, *miR-219*, *miR-26a*, *miR-1b-1* and *miR-30c-1* are at least three times differentially expressed in African Americans compared to Caucasians. Interestingly of the five miRNAs highlighted by Calin and Croce, significant increases in *miR*-26a expression has been reported in perineural invasion (PNI)[11] and in advanced prostate cancer.[9] The purpose of this study to determine the expression levels of *miR*-26a associated with African American males with prostate cancer.

Herein we report that African American prostate cancer cell lines have increased *miR*-26a expression when compared to Caucasian cells lines of similar stages. Furthermore, endogenous *miR*-26a expression increases in the more advanced prostate cancer cell lines, in both African American and Caucasian, which is akin to the previously reported clinical samples of prostate adenocarcinoma tissue.[9]

Materials and Methods

To determine the expression pattern of *miR*-26a, we assembled a panel of non-malignant and malignant African American and Caucasian prostate cell lines representing various clinical stages of prostate progression. The tissues were obtained from radical prostatectomy specimens according to Walter Reed Medical Center and Uniformed Service University of the Health Sciences Internal Review Board Protocol. The clinical and pathological features of the patients from whom these cell lines were obtained are shown in Table 1. The tumor tissue RC77T was obtained from a 62-year-old African American patient who had clinical stage T3c adenocarcinoma with poor differentiation (Gleason Score 7). The presence of prostatic adenocarcinoma was confirmed under light microscopy. Fresh prostatectomy specimens were obtained under sterile conditions by an experienced pathologist. Tumor tissue on gross inspection was dissected separately for the purpose of generating a cell culture. The primary cells were transduced with HPV-16 E6E7 genes for immortalization and cultured in Keratinocyte Serum-Free Medium (KGM) (Cat. 10724–011, LifeTechnologies, Gaithersburg, Md., USA), supplemented with bovine pituitary extract (BPE), recombinant epidermal growth factor (rEGF), 1% (v/v) penicillin-streptomycin-neomycin (PSN) antibiotic mixtures and 1% (v/v) amphotericin B (KGM) (LifeTechnologies, Gaithersburg, Md, USA). Malignant Caucasian RC-92a/hTERT cells

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were maintained in KGM as previously described.[12] Non-malignant Caucasian prostate epithelial cells (PrEC) were obtained from Lonza, Switzerland, and maintained in PrEGM (Clonetics, Lonza, Switzerland). The African American androgen independent and meta-static MDA-PCa- 2b cells were cultured in F12K medium as previously described.[13] The Caucasian androgen independent and metastatic PC-3 and C42-B prostate cancer cell lines were maintained in T-medium as previously described.[14]

miRNA Isolation and Real-time Quantitative PCR

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. RNUB6 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 UUCAAGUAAUCCAGGAUAGGC 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 $2^{-\Delta Ct}$ 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 chromosome 3p22.3/12q14.[15] 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 13.3 fold increase *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 and C4-2b (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).

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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)[16] and MDA-PCa 2 (from a single metastatic location),[13] 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,[10] and the follow-up report from these same investigators that matched tumors have increased *miR*-26a as well.[15] 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 Target-Scan 3.0. (http://www.targetscan.org/), utilize different algorithms and ranking criteria[17] to generate a partially overlapping set of gene candidates for a given miRNA. Interesting, *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 tissue[10] 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.

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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

1. Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. CA:A Cancer Journal for Clinicians. 2004; 54(1):8–29. [PubMed: 14974761]
2. Bennett CL, Price DK, Kim S, et al. Racial variation in CAG repeat lengths within the androgen receptor gene among prostate cancer patients of lower socioeconomic status. J Clin Oncol. 2002; 20(17):3599–3604. [PubMed: 12202660]
3. Irvine RA, Yu MC, Ross RK, Coetzee GA. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. Cancer Res. 1995; 55(9): 1937–1940. [PubMed: 7728763]
4. Shuch B, Mikhail M, Satagopan J, et al. Racial disparity of epidermal growth factor receptor expression in prostate cancer. J Clin Oncol. 2004; 22(23):4725–9. [PubMed: 15570072]
5. Dalmay T, Edwards DR. MicroRNAs and the hallmarks of cancer. Oncogene. 2006; 25(46):6170–6175. [PubMed: 17028596]
6. Barad O, Meiri E, Avniel A, et al. MicroRNA expression detected by oligonucleotide micro-arrays: system establishment and expression profiling in human tissues. Genome research. 2004; 14(12): 2486–2494. [PubMed: 15574827]
7. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci, USA. 2006; 103(7):2257–2261. [PubMed: 16461460]
8. Chuang JC, Jones PA. Epigenetics and micro-RNAs. Pediatric Research. 2007; 61(5 Pt 2):24R–9R.
9. Porkka KP, Pfeiffer MJ, Waltering KK, et al. MicroRNA expression profiling in prostate cancer. Cancer Research. 2007; 67(13):6130–6135. [PubMed: 17616669]
10. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nature Rev. 2006; 6(11):857–66.
11. Prueitt RL, Yi M, Hudson RS, et al. Expression of microRNAs and protein-coding genes associated with perineural invasion in prostate cancer. The Prostate. 2008; 68(11):1152–64. [PubMed: 18459106]
12. Gu Y, Li H, Miki J, et al. Phenotypic characterization of telomerase-immortalized primary non-malignant and malignant tumor-derived human prostate epithelial cell lines. Experimental Cell Research. 2006; 312(6):831–43. [PubMed: 16413016]
13. Navone NM, Olive M, Ozen M, et al. Establishment of two human prostate cancer cell lines derived from a single bone metastasis. Clin Cancer Res. 1997; 3(12 Pt 1):2493–500. [PubMed: 9815652]
14. Huang WC, Xie Z, Konaka H, et al. Human osteocalcin and bone sialoprotein mediating osteomimicry of prostate cancer cells: role of cAMP-dependent protein kinase A signaling pathway. Cancer Research. 2005; 65(6):2303–13. [PubMed: 15781644]

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15. Ambs S, Prueitt RL, Yi M, et al. Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer Research. 2008; 68(15):6162–6170. [PubMed: 18676839]
16. Koochekpour S, Maresh GA, Katner A, et al. Establishment and characterization of a primary androgen-responsive African-American prostate cancer cell line, E006AA. The Prostate. 2004; 60(2):141–152. [PubMed: 15162380]
17. Krek A, Grun D, Poy MN, et al. Combinatorial microRNA target predictions. Nature Genetics. 2005; 37(5):495–500. [PubMed: 15806104]
18. Kulshreshtha R, Ferracin M, Wojcik SE, et al. A microRNA signature of hypoxia. Molecular and Cellular Biol. 2007; 27(5):1859–67.
19. deVere White RW, Deitch AD, Jackson AG, et al. Racial differences in clinically localized prostate cancers of black and white men. J Urol. 1998; 159(6):1979–1982. discussion 82–3. [PubMed: 9598502]
20. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nature Rev. 2002; 2(8):563–572.
21. Yates CC, Shepard CR, Stolz DB, Wells A. Co-culturing human prostate carcinoma cells with hepatocytes leads to increased expression of E-cadherin. Br J Cancer. 2007; 96(8):1246–1252. [PubMed: 17406365]

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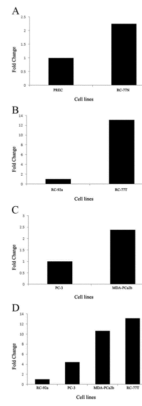

Fig 1.

Expression of hsa-mir-26a in African American vs Caucasian prostate cell lines. *(A)* qRT-PCR of non-malignant African American RC-77N was compared to Caucasian PrEC cells *(B)* malignant RC-77T was compared to RC-92a, *(C)* metastatic MDA-Pca2b was compared to PC-3 cells (D) qRT-PCR was performed to determine relative expression levels of RC-92a, PC-3, MDA-PCa2b and RC-77T. Experiments were preformed in triplicates

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Table 1

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

Cell line	Age	Race	Morphology	Clinical Stage	Tumor Grade	Gleason Score
RC77N	62	AA	Epithelial	Non-malignant	NA[c]	NA[c]
RC77T	62	AA	Epithelial	Primary adenocarcinoma	Poorly differentiated	7
MDA-2Pca-2b	63	AA	Epithelial	Adenocarcinoma Metastatic tumor	NA	NA
PrEC	59	White	Epithelial	Non-malignant	NA[c]	NA[c]
RC-92a	57	White	Epithelial	Primary adenocarcinoma	Well-differentiated	3+3
PC-3	62	White	Epithelial	Metastatic adenocarcinoma	Undifferentiated	NA[c]

AA = African American.

NA = not applicable.

NA[c] = 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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Table 2

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

Cell Line	qRT-PCR fold change	*P* value
Non-malignant		
RC-77N	2.25	*P*<.001
Malignant		
RC-77T	13.13	*P*<.001
Metastatic		
MDA-Pca2b	2.38	*P*<.001

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).