ORIGINAL ARTICLE

A health disparities study of MicroRNA-146a expression in prostate cancer samples derived from African American and European American patients

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ABSTRACT

Considering the prevalence of prostate cancer all over the world, it is desired to have tools, technologies, and biomarkers which help in early detection of the disease and discriminate different races and ethnic groups. Genetic information from the single gene analysis and genome-wide association studies have identified few biomarkers, however, the drivers of prostate cancer remain unknown in the majority of prostate cancer patients. In those cases where genetic association has been identified, the genes confer only a modest risk of this cancer, hence, making them less relevant for risk counseling and disease management. There is a need for additional biomarkers for diagnosis and prognosis of prostate cancer. MicroRNAs are a class of non-protein coding RNA molecules that are frequently dysregulated in different cancers including prostate cancer and show promise as diagnostic biomarkers and targets for therapy. Here we describe the role of micro RNA 146a (miR-146a) which may serve as a diagnostic and prognostic marker for prostate cancer, as indicated from the data presented in this report. Also, a pilot study indicated differential expression of miR-146a in prostate cancer cell lines and tissues from different racial groups. Reduced expression of miR-146a was observed in African American tumor tissues compared to those from European Whites This report provides a novel insight into understanding the prostate carcinogenesis.

Key Words: Biomarker, MicroRNA, Noncoding RNA, Prostate cancer

1. INTRODUCTION

Although biomarkers exist for prostate cancer diagnosis, but either they have poor sensitivity and specificity or they cannot detect prostate cancer early enough to make strategies for proper treatments. Only prostate specific antigen (PSA) is a biomarker which is used extensively in the clinic and it can be only used in those cases where the levels of PSA are more than 4 ng/ml. Different forms of PSA (free PSA, total PSA, PSA velocity) have been characterized but the advantage of one over others is not convincing.[1]

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Cancer is a genetic and epigenetic disease and epigenetic regulation has been observed in all major tumor types studied to date including prostate cancer.[2–4] The four major components of epigenetics are DNA methylation, histone modifications, non-coding RNA (mostly microRNAs) expression and chromatin modulation.[1, 5–9] In the last few years, tremendous progress has been made in understanding the biogenesis of miRNAs and their role in altering the translation of messenger RNA by binding to them or degrading them. Compared to mRNAs, microRNAs are shorter in length and have a longer half-life (due to their secondary structure and size) which makes them stable and potentially reliable as clinical diagnostic and prognostic biomarkers.[10, 11] Whether the miRNAs act as tumor suppressors or oncogenes, dysregulated miRNAs allow cells to escape from regulatory control and contribute in tumor formation.[12, 13] Enabling early detection and aggressive treatment of rapidly recurrent and refractory phenotypes would reduce disease mortality. Dysregulated miRNAs are also potentially viable novel targets of prostate cancer. miRNAs either comes in the circulation as free-floating bodies or they are delivered via exosomes (small molecules with bipolar membrane).[14, 15] Recently, the roles of microRNAs (miRNAs) in the development and progression of cancers including prostate cancer became one of the major hot topics in cancer research. Emerging studies have been focused on the biosynthesis, function, and the molecular regulation of miRNAs in normal physiological and pathological conditions in chronic diseases including different types of cancers. The emerging evidence also shows that the tiny molecule, the miRNA, has huge impact on cell growth, differentiation, and apoptosis through the regulation of specific gene expression. The miRNAs are a naturally occurring, non-coding small (commonly 19-22 nucleotide long) RNAs which are highly conserved across different species and highly specific for developmental stages. In vivo, miRNAs are biosynthesized from differential miRNA genes and matured from pri-miRNA and pre-miRNA to miRNA. In cells, RNA polymerase II is necessary for constructing miRNA chains. Firstly, using miRNA gene as templates RNA polymerase II synthesizes miRNA precursors (pri-miRNAs) which have comparatively longer sequences. Following this transcription, Drosha and Dicer cut the pri-miRNA to produce pre-miRNA duplex which moves from nucleus to cytosol. In cytosol, the miRNA duplex is dissociated and the single strand miRNA becomes the mature miRNA. The mature miRNA is then incorporated in the complex known as RNA-induced silencing complex (miRISC). In the majority of cases, by binding of miRNA to the 3'-UTR of target mRNAs through partial complementarity, miRNA induces the process for silencing the protein expression of the target genes through translational repression or mRNA degradation. Because miRNAs control cell growth, differentiation and apoptosis, the alterations in the expression of miRNAs has been correlated with cancer development and progression. By conducting miRNA expression profiling, the aberrant expression of miRNAs has been found in various cancers including prostate cancer. In prostate cancer cells, several miRNAs act as oncogenic mediators which inhibit the expression of tumor suppressors, resulting in development and progression of pancreatic cancer. On the other hand, some miRNAs function as tumor suppressors which down-regulate the expression of oncogenes, and thereby inhibit cancer growth and aggressiveness. Significantly increased levels of oncogenic miRNAs and decreased levels of tumor suppressive miRNAs have been found in prostate pre-cancerous and cancerous cells , suggesting that the aberrant expressions of miRNAs together with the disorders of cellular signal transduction constitute the molecular basis of prostate cancer development and progression. Since each miRNA has hundreds of different conserved or non-conserved targets, the alterations in the level of a specific miRNA could cause significant alterations in the expression of many genes, and thereby promote aberrations in multiple cellular signal transduction pathways, resulting in development and progression of prostate cancer. Therefore, targeting these miRNAs could provide an efficient approach for prevention or treatment of prostate cancer through the regulation of multiple genes. The miRNA family of mir-146a and mir-146b was first found in mouse, which shared -91% homology to humans. The identical sequences were also identified in human, indicating that this miRNA family might target the same genes conserved in humans (http://microrna.sanger.ac.uk). One of the major target genes of mir-146a, ROCK1, was recently reported to be highly involved in the transformation of HRPC and metastasis in vivo and in HRPC-derived PC3 cells. Research showed miR-146a was down-regulated in androgen-independent prostate cancer(AIPC) tissues and cell lines compared to that in the androgen-dependent (ADPC) tissues. In Memorial Sloan Kettering Cancer Center MSKCC clinical data analyses, it was found that miR-146a was under expressed in metastatic prostate cancer tissues and those with Gleason score > 8, moreover, low level of miR-146a represented a high biochemical relapse rate after radical prostatectomy. In the functional analyses, transfected miR-146a mimics into PCa cell lines showed apoptosis. In mechanic analyses, it was found that miR-146a inhibited the basal level of Rho-associated, coiled-coil containing protein kinase 1 (ROCK1) expression by targeting its 3'UTR and an inverse correlation of expression between miR-146a and ROCK1 was observed. Moreover, caspase 3 activity was stimulated by miR-146a

overexpression. Thus miR-146a plays a significant role in PCa progression and mortality.

African Americans (AA) have a higher incidence of PCa occurrence and mortality in USA than other ethnic groups like European descent Americans(UA).In this Health Disparities research, we were interested to study the role of non coding RNA miR-146a in PCa cell lines and biopsy samples obtained from AA and UA patient cohorts. We used Microarray analysis and Real Time PCR techniques to analyze our results.

2. METHODS AND MATERIALS

2.1 Cell culture and maintenance

Cell culture and maintenance were described in our previous publication.[?] C42b (UA PCa), RWPE1 (Healthy prostate cell line), MDA (AA PCa), EOO6AA cell lines were obtained from the American Type Culture Collection. The cell lines were grown and maintained in Roswell Park Memorial Institute Medium (RPMI 1640). Cells were stored at 5% CO_3 in 25cm^2 filter cap flasks in a laboratory CO_3 incubator and were additionally maintained with 10% Fetal Bovine Serum (FBS) and 100μl/ml antibiotic/ anti mycotic (penicillin/ streptomycin/ amphotericin B). RWPE1 are normal prostate cell line, C42b is from European American derived prostate cancer line and MDA is African American derived prostate cancer cell line.

2.2 Patients and prostate tissue specimen collection

After obtaining institutional review board approval, retrospective archival pre-treatment PCa tissues and matched adjacent normal tissues were obtained from Biospecimen Core of Karmanos Cancer Institute (KCI) collected from patients who underwent radical prostatectomy at KCI. We also obtained PCa tissue specimens from Henry Ford Health System (HFHS), Detroit, Michigan. Patients' clinical characteristics were obtained from the hospital database including race. Pathological features were ascertained from microscopic evaluation of tumor slides by pathologists both at KCI and at HFHS. Gleason score (grade) was obtained in each case from the clinical database.

2.3 Real-time RT-PCR

For testing the miRNA levels, the total RNA from cells and tissues were isolated using the miRNeasy Mini Kit (Qiagen) and the DNA was removed using an RNase-free DNAase kit (Qiagen). 20 ng of RNA were reverse transcribed into cDNA using a Universal cDNA Synthesis Kit (Exiqon, Woburn, MA) according to the manufacturer's instruction. Real time PCR was performed using specific miRNA primers (Exiqon) to quantify miRNA expression by using SYBR® Green RT-PCR Reagents (Applied biosystems). The relative amount of miRNA was normalized to the expression of RNU1a1.

2.4 Microarray analysis and gene expression profiling RNA sample preparation

Total cellular RNA was isolated from the human prostate cancer exposed human Macrophage cells using Trizol (Invitrogen, CA, USA). The RNA quantity was analyzed using the Nano Drop ND1000 (SOP N° TAL009) and RNA quality checked using a Bio-analyzer 2,100 (Agilent Technologies, CA, USA). Sample amplification was performed with 200 ng of total RNA using Agilent Technologies Quick Amp Labeling Kit One Color to generate complementary RNA (cDNA) for oligo microarrays. cDNA microarray analysis was processed using a Whole Human Genome Oligonucleotide Microarray (G4112A, 41,000 genes; Agilent Technologies, CA, USA) according to the manufacturer's instructions.

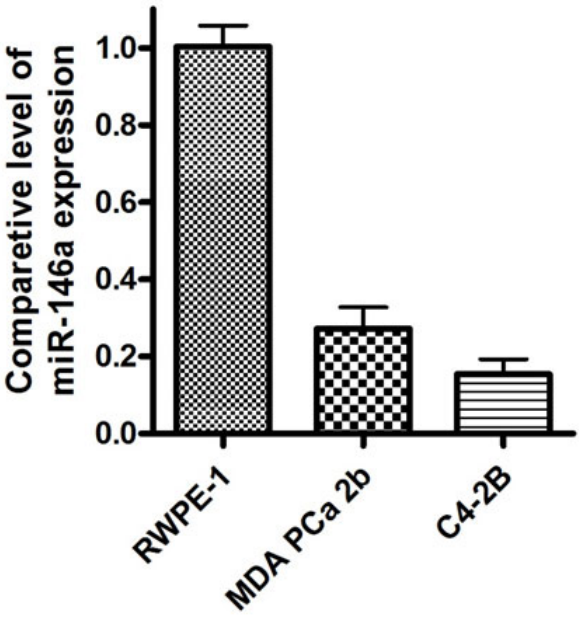

Figure 1. Decreased expression of miR 146 a in PCa cell lines MDA and C42b in comparison to the normal RWPE-1 prostate cell line

2.5 Microarray hybridization

To prepare samples for microarray analysis, slides were hybridized in buffer containing fluorescence labeled cDNA at 60°C, 17 h using HS Pro hybridization station. Slides were washed once with 63 × SSPE buffer containing 0.005% N-lauryl sarcosine, 1 min at room temperature followed by a 1 min wash using pre heated (37°C) 0.06 × SSPE buffer containing 0.005% N-lauryl sarcosine. The final slide wash was performed for 30 sec using acetonitrile.

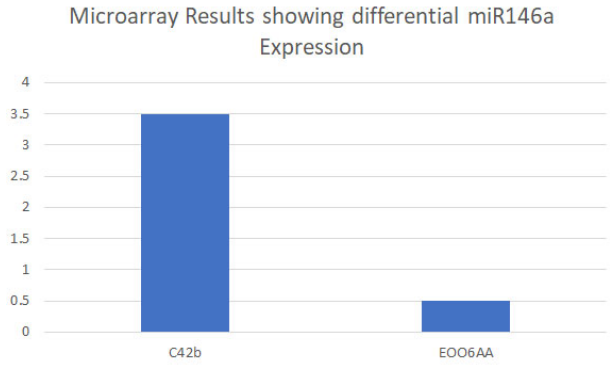

Figure 2. Microarray analysis of RNA expression from C42b(CA) and EOO6AA(AA) cell lines showing higher fold change in prostate cancer cells derived from CA patient than AA patient. X axis- Cell lines, Y axis-Fold Change

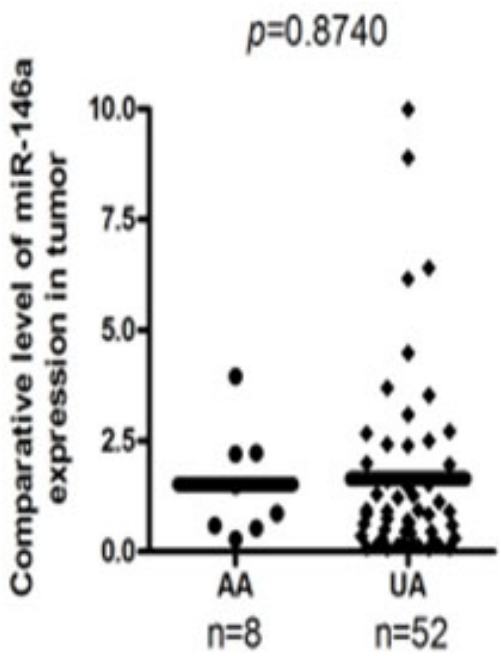

Figure 3. European American (UA) patients had a higher expression of miR-146a in tumor compared with African American (AA) patients. Total RNAs were extracted from prostate cancer tissues. The patients are AA or UA with all grades of prostate cancer. The expression of miR-146a in tumor was accessed by real-time RT-PCR assay(Exipon). The expression level of miR-146a wasnormalized with the level of RNU1a1.

2.6 Image and data extraction

Fluorescence signals from hybridized microarrays were detected using an Agilent and DNA microarray scanner with a resolution of 5l M and using Agilent Feature Extraction Software (FES). FES determines feature intensities and normalized ratios by linear LOWESS with background subtraction, rejects outliers and calculates statistical confidences (*P*-values). Hybridization signals with *P* value less than 0.001 were considered significant. Only genes differentially expressed in the three repeat experiments were considered as relevant genes.

3. Results

Levels of miR-146a was found reduced in prostate cancer cell lines compared to normal cell lines (see Figure 1). Our Microarray analysis with AA cell line EOO6AA comparison to CA cell line C42b showed increased miR146a expression in C42b RNA expression analysis (see Figure 3). Investigating whether similar results were seen in patient samples, expression of miR-146a was analyzed in prostate cancer tissues and compared in tissue biospecimens, the same experiment was conducted in biospecimens from African American and European White subjects (see Figure 2). Reduced expression of miR-146a was observed in African American tumor tissues compared to those from European Whites.

4. Discussion

Deregulation of microRNAs resulting in abnormal expression of target genes is considered a significant factor in carcinogenesis process. miR-146a expression is decreased in various cancers, such as NK/T cell lymphoma,[15] gastric cancer,[17] castration-resistant prostate cancer,[18] head and neck squamous cell carcinoma,[19] hepatocellular carcinoma,[20] penile squamous cell carcinoma[21] and non-small cell lung cancer.[22] Enforced expression of miR-146a suppresses cell proliferation and invasion[15,17–20,22–25] and angiogenesis via targeting EGFR,[18] RAF6,[20] CCND1/2,[22] Notch1[23] or Rac1,[24] induces cell apoptosis[15,17,25] and enhanced chemosensitivity.[15] Therefore, miR- 146a may function as a tumor suppressor gene in PCa.[26,27] miR-146a has a critical role in the process of AIPC prostate cancer cells apoptosis through regulation of ROCK/Caspase 3 pathway.[28–30] Targeting this pathway may be a promising therapeutic strategy for future personalized anti-cancer treatment.[30–32] Our study showed in PCa cell lines miR 146a was downregulated in comparison to healthy prostate cells, also in the small cohort of AA and UA patients PCa samples we analyzed by Real Time PCR, miR 146a was consistently less expressed in AA patients which was substantiated by similar data obtained by microarray gene expression analysis of C42b(CA) and EOO6AA(AA)cell lines. Thus downregulation of this noncoding RNA could be one of the reasons for increased malignant form of the disease in the AA population, even though a larger sample size with longitudinal cohort study will be needed for any conclusive evidence.

Conflicts of Interest Disclosure

The authors declare no conflict of interest

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