MiR-139-5p is Increased in the Peripheral Blood of Patients with Prostate Cancer

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Key Words
MiR-139-5p • Prostate cancer • Benign prostatic hyperplasia • Biomarker • Peripheral whole blood

Abstract
Background: Emerging evidence suggested that microRNAs (miRNAs) play a causal role in cancer tumorigenesis. Aberrant expression of miRNA (miR)-139-5p has been observed in various types of cancers. The present study evaluated the relationship between miR-139-5p expression levels and prostate cancer (PCa), to assess the feasibility of using peripheral blood miR-139-5p as a potential non-invasive biomarker for PCa. Methods: Total RNA was extracted from peripheral whole blood samples from 45 PCa patients, 45 benign prostatic hyperplasia (BPH) patients and 50 healthy controls (HC). The expression of miR-139-5p was assessed by reverse transcription quantitative polymerase chain reaction. Results: MiR-139-5p in peripheral blood was significantly higher in PCa patients than in patients with BPH and HC individuals (P<0.001). Higher miR-139-5p expression was observed to be associated with certain clinicopathological parameters, including PSA>20ng/ml (P<0.05), pathological tumor stage 3/4 (P<0.05) and Gleason score >7 (P<0.01). A receiver operating characteristic (ROC) curve analysis revealed that miR-139-5p distinguished PCa patients from BPH patients [area under the curve (AUC), 0.936; 95% CI, 0.878-0.993; P<0.001]. Conclusions: Peripheral blood miR-139-5p may be utilized as a potential novel non-invasive biomarker for PCa screening.

Introduction

PCa is the most commonly diagnosed malignant tumor in male and the second leading cause of cancer-associated deaths in western countries, with increasing mortality and incidence rates [1]. Currently, serum prostate-specific antigen (PSA) is the standard diagnostic biomarker for PCa, which exhibits increased levels in patients with PCa. According
to the different level of serum PSA (PSA <10 ng/ml, PSA 10-20 ng/ml and PSA >20 ng/ml), combined with tumor stage and Gleason score, PCa can be divided into three different risk groups (low-risk, intermediate-risk and high-risk) [2]. However, the level of serum PSA could be increased in various diseases, including trauma, prostatitis and BPH [3], which usually leads to overdiagnosis and overtreatment [4]. Furthermore, the PSA measurement is insufficient to identify clinical insignificant PCa. Urinary prostate cancer antigen 3 (PCA3) has been used as a non-invasive diagnostic biomarker for PCa screening, and the nomograms base on clinical parameters is also helpful, but it is not sufficient to distinguish PCa patients from healthy individuals [5-7]. Therefore, it is urgent to develop more effective non-invasive biomarkers for screening the populations at risk.

MiRNAs belong to a class of highly conserved short non-coding RNAs, which regulate gene expression by binding to the 3′ untranslated region (3′ UTR) of target messenger RNAs (mRNAs) [8, 9]. MiRNAs play crucial roles in various cellular functions, including cell differentiation, proliferation and apoptosis [10]. Several evidences suggest that aberrant expression of miRNAs contributes to tumor proliferation, apoptosis, metastasis, invasion and anti-tumor drug resistance [11-13]. A variety of miRNAs have been found to be dysregulated in PCa [5, 6, 14]. Moltzahn et al found that miR-93, miR-106a and miR-24 were higher in the patients with early stage PCa immediately before prostatectomy than the healthy males [15]. According to Bryant et al, 12 miRNAs were altered in the circulation system of 78 patients with PCa compared with 28 healthy males, and miR-107 was one of the greatest changed biomarkers in this study [16].

In particular, miR-139-5p is one of the most common cancer-associated miRNAs, which has been reported as tumor suppressor in several malignancies, such as breast cancer [17], colorectal carcinoma [18], hepatocellular carcinoma [19], parathyroid carcinoma [20], and so on. On the contrary, overexpression of miR-139-5p has been recently correlated with granulose cells and bladder carcinoma [21]. However, the expression of miR-139-5p in PCa remains unclear.

Therefore, the present study aims to evaluate the expression of miR-139-5p in the peripheral whole blood of PCa, BPH patients, and in healthy males, thereby investigating the potential use of blood miR-139-5p as a non-invasive PCa diagnostic biomarker.

Materials and Methods

Patients and blood samples

All peripheral whole blood samples of PCa, BPH patients, and healthy individuals were obtained from the Department of Urology, Beijing Hospital (Beijing, China) between June 2014 and January 2016. The application of patient-derived materials was approved by the Research Ethics Committee of Beijing Hospital, and written consent was obtained from all patients. No patients received androgen deprivation therapy or radiotherapy. The characteristics of the patients were shown in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prostate cancer</th>
<th>Benign prostatic hyperplasia</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total subjects: n</td>
<td>45</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>Age, years; mean (range)</td>
<td>70 (55-83)</td>
<td>69 (55-81)</td>
<td>70 (56-81)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4±2.01</td>
<td>24.1±3.34</td>
<td>25.5±2.38</td>
</tr>
<tr>
<td>PSA, ng/ml; n (%)</td>
<td>&lt;10 21 (46.7)</td>
<td>45 (100.0)</td>
<td>50 (100.0)</td>
</tr>
<tr>
<td></td>
<td>10-20 7 (15.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>&gt;20 17 (37.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gleason score: n (%)</td>
<td>&gt;7 (low) 10 (22.2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>7 (intermediate) 26 (57.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>&gt;7 (high) 9 (20.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tumor stage: n (%)</td>
<td>pT2 29 (64.4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>pT3 16 (35.6)</td>
<td>—</td>
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RNA extraction

The total RNA from the whole blood samples (5 ml) collected in tubes containing EDTA was extracted with RNAVozil LS (Vigorous Biotechnology, Beijing) rigorously according to the manufacturer’s instructions. The concentration and the purity of the RNA samples were assayed by absorbent density analysis on OD$_{260}$/OD$_{280}$.

Reverse transcription (RT) and quantitative (q) polymerase chain reaction (PCR)

For synthesis of cDNA of the specific miR, 1μg of the total RNA was reversely transcribed using Taq-Man MicroRNA Reverse Transcription Kit (Applied Biosystems) with specific primers for miR-139-5p and U6 (Shanghai Sangon Technology). To quantify the miR-139-5p, a quantitative real-time PCR assay was performed using SYBR Green Supermix (Bio-Rad) in a BIO-RAD iCycleriQ real-time PCR detection system. The PCR amplifications were performed in a 10μl reaction system containing 5μl SYBR Green Supermix, 0.4μl forward primer (10nM), 0.4μl reverse primer (10nM), 2.2μl ddH2O and 2μl template cDNA. The thermal cycling conditions were a hot start step at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The relative level of miR-139-5p was determined using the 2-delta delta Ct analysis method. We choose U6 as the endogenous control. Nucleotide primers used for reverse transcription were as follows (5’-3’): miR-139-5p, CGT ATC CAG TGC AGG GTC CGA GGT ATT CGC ACT GGA TAC GAC ACT GGA; U6, TCG TAT CCA GTG CAG GGT CCG AGG TAT TCG CAC TGG ATA CGA CAA ATAT G.

The primers used for real-time PCR were as follows (5’-3’): miR-139-5p forward, GCT CTA CAG TGC ACG TGT C; U6 forward, GCG TCG TGA AGC GTT C; Universal reverse primer, GTG CAG GGT CCG AGG T.

Statistical analysis

Data are presented as mean ± SD. Differences were carried out with Student’s t test. ROC curves were used to assess miR-139-5p as a biomarker, and the AUC was reported. P < 0.05 was considered as statistically significant difference.

Results

Patients characteristics

The demographics and clinical characteristics of the HC, BPH patients and PCa patients were outlined in Table1. The age distribution was similar in the three groups (P>0.05). Serum PSA levels were significantly higher in PCa patients compared with that in BPH patients and HC individuals (P<0.001).

Elevated expression of miR-139-5p in peripheral blood of PCa patients compared with BPH patients and healthy individuals

The level of miR-139-5p was examined in the peripheral blood of PCa, BPH patients and HC subjects using real-time PCR. The expression of miR-139-5p was significantly up-regulated in the peripheral blood of patients with PCa compared with that of BPH patients.

Fig. 1. Elevated expression of miR-139-5p in peripheral blood of prostate cancer (PCa) patients compared with benign prostate hyperplasia (BPH) patients and healthy individuals (healthy controls, HC). The levels of miR-139-5p was measured in peripheral blood samples from PC patients (n=45), BPH patients (n=45) and healthy control individuals (n=50). Data represent the mean ± S.D. * P<0.05; ** P<0.01; *** P<0.001 (vs. BPH patients and controls).
and HC individuals (P<0.001) (Fig. 1). The relative expression of miR-139-5p was 5.3±2.8 for PCa patients, 1.4±0.8 for BPH patients and 1±0.8 for healthy controls.

**Clinicopathological parameters changes are accompanied by up-regulation of miR-139-5p in peripheral blood of prostate cancer patients**

PCa patients with more aggressive tumors exhibited significantly higher miR-139-5p expression compared with that of patients with less aggressive tumors. The mean relative miR-139-5p level in the group of patients with a Gleason score <7 was 1±0.5, and increased...
progressively to 1.6±0.8 in patients with a Gleason score of 7, and to 2.2±1.1 in patients with a Gleason score >7 (Fig. 2A). In addition, miR-139-5p expression was significantly higher in patients at an advanced stage of disease: Patients with a pathological tumor (pT) stage of 3 or 4 exhibited a mean relative miR-139-5p expression level of 1.9±1.0, which was significantly increased compared with patients of pT stage 1 or 2 (1.0±0.7) (P<0.05) (Fig. 2B). The levels of miR-139-5p in patients with PSA<10, PSA=10-20 and PSA>20 was respectively 1±0.8, 1.6±0.9 and 2.1±1.0 (Fig. 2C).

The expression of miR-139-5p may be regarded as a potential diagnostic tool

As miR-139-5p was differentially expressed in the peripheral blood of PCa patients, BPH patients and HC, peripheral blood miR-139-5p might be a promising marker. Based on an ROC analysis, blood miR-139-5p was able to distinguish PCa patients from healthy controls (AUC, 0.915; 95% CI, 0.846-0.984; P<0.001; Fig. 3A), suggesting miR-139-5p can distinguish PCa patients from BPH patients (AUC, 0.936; 95% CI, 0.878-0.993; P<0.001; Fig. 3B).

Discussion

PCa is the most commonly diagnosed malignance in male. The main parameters for PCa diagnosis are PSA testing, digital rectal examination (DRE), PCA3, multiparametric magnetic resonance image (mpMRI) and Gleason score [5]. However, the diagnostic tools are relatively limited. MiRNAs belong to a type of small non-coding RNAs that are widely involved in both physiological and pathological condition [22-24]. For instance, miR-125b [25] and miR-449a [26] are respectively involved in the physiological condition of apoptosis and autophagy. At the same time, miR-135a [27] and miR-21 [28] are respectively involved in the pathological process of tumor and insulin resistance. It has been widely acknowledged that any disruption of miRNAs may cause dysregulation in cellular homeostasis and further lead to oncogenesis [29]. Therefore, the investigation of miRNAs may shed light on the understanding of PCa progression and be helpful to identify potential biomarkers.

MiR-139-5p is one of the most important miRNAs that has been shown to be dysregulated in a variety of cancers, including breast cancer, gastric cancer [30, 31]. In the current study, we firstly explored the expression of miR-139-5p in peripheral blood and determined that it was significantly higher in PCa patients than in patients with BPH and HC individuals, which showed a strong association between PCa and miR-139-5p expression. More importantly, the more high-risk stage, the higher expression of the miR-139-5p was found. Much higher expression of peripheral blood miR-139-5p was detected in PCa patients with more advanced stage (pT3/4) and more aggressive tumors (Gleason score >7), suggesting that peripheral blood miR-139-5p may be tightly associated with progression of PCa. In addition, the ROC analysis revealed that peripheral blood miR-139-5p was able to distinguish patients with PCa from BPH patients and HC, respectively.

Collectively, these results suggested a role for miR-139-5p in the pathogenesis of PCa, and indicated miR-139-5p as a non-invasive biomarker with high specificity and sensitivity for PCa detection.

Previous studies have shown that miR-139-5p can act as either tumor oncogenes or suppressors in several types of cancers. Yamashita et al observed that miR-139-5p can decrease tumor growth by inhibit the expression of HER2 in gastric cancer cell lines [32]. Xu et al exhibited that miR-139-5p functioned as a tumor suppressor by targeting insulin-like growth factor 1 receptor in human non-small cell lung cancer [33]. Zhang et al found that miR-139-5p inhibited the viability, invasion and migration of breast cancer cells by targeting notch1 [34]. The function of a given miRNA is determined by the relative availability of the downstream target mRNAs, as such the same miRNA could have a dual role in different tissues, specifically cancers of different cellular origin [35]. MiRNAs have been shown to function as either tumor suppressors or oncogenes depending on the cell or cancer type in which they are expressed. For example, mir-155 may function as a tumor suppressor in...
pancreatic cancers [36], while in B-cell lymphomas it has oncogenic activity [37]. Another example of this is miR-125b, which has been shown to be a tumor suppressor in breast and ovarian cancer and to be a tumor oncogene in PCa [38]. So this situation is not unique to miR-139-5p.

In conclusion, the present study has a limited number of experimental samples, which may restrict the statistical power of the study. Therefore, several large prospective studies are required to confirm the role of miR-139-5p in PCa. Despite this disadvantage, this study provides a novel potential non-invasive biomarker for PCa detection.

**Disclosure Statement**

The authors declared no conflict of interest.

**References**