MicroRNA and messenger RNA As Potential Urinary Biomarkers in Prostate Cancer

Keywords: Prostate cancer; Biomarker; miR-21-5p; PDCD-4

Abstract

Introduction: Prostate Cancer (PCa) is the fifth leading cause of death worldwide and these are predominantly common cancer in men. Worldwide, it was estimated that around 1,276,106 newly diagnosed PCa were reported in 2018 [1]. The diagnosis of suspected PCa is made when the abnormality from the Digital Rectal Examination (DRE) and elevated Prostate Specific Antigen (PSA) present [2]. PSA value of more than 4 ng/ml is an indication for prostate biopsy examination and this border line value has a positive predictive value of only 37%, and a negative predictive value of 91% [2]. Therefore, rigorous studies searching for new biomarkers with non-invasive methods, are carried out, one of which is urinary biomarker as an early detection and predictors of PCa prognosis. microRNA (miRNA) and messenger RNA (mRNA) has proven to have important roles in various oncogenic processes.

Material and methods: Urine samples collected from 145 patients were examined. 45 patients diagnosed with Benign Prostate Hyperplasia (BPH) and 100 patients diagnosed with PCa. Urine samples were collected from each patient and examined in the molecular laboratory. The gene expression were analyzed using qPCR analysis using the qPCR CFX 96 thermocycler Bio-Rad.

Results: The expression of miR-21-5p was higher in BPH group compared to PC a groups, both non-metastatic and metastatic, with p-values of 0.004 and 0.017, respectively. BPH showed the highest mRNA expression of PDCD-4.

Conclusion: The overexpression of miR-21-5p shown in this study could be a potential non-invasive diagnostic tool for patients with PCa. The lower expression of miRNA-21 in non-metastatic compared to metastatic PCa group could be potential prognostic biomarker in PCa.

Introduction

Prostate Cancer (PCa) is the fifth leading cause of death worldwide and the second most common cancer in men. Worldwide, it was estimated that around 1,276,106 newly diagnosed PCa were reported in 2018 [1]. The diagnosis of suspected PCa is made when the abnormality from the Digital Rectal Examination (DRE) and elevated Prostate Specific Antigen (PSA) present [2]. PSA value of more than 4 ng/ml is an indication for prostate biopsy examination and this border line value has a positive predictive value of only 37%, and a negative predictive value of 91% [2]. Therefore, rigorous studies searching for new biomarkers with non-invasive methods that have higher specificity than PSA and can be used as an early detectors as well as prognostic predictors of PCa, have emerged [3].

microRNA (miRNA) is a non-coding RNA molecule which regulates gene expression and influences both the stability and the efficiency of target messenger RNA (mRNA) [4]. It has been proven that specific miRNA plays a key role in various oncogenic processes, including angiogenesis, epithelial-mesenchymal transition, and metastasis. Micro-RNA was suggested to be a potential biomarker in both serum and urine samples of patients with PCa [3].

miRNA-21 (miR-21) is a specific miRNA which is frequently up-regulated in cancer and has many targets of tumor suppressors, such as Phosphatase And Tensin Homolog (PTEN), Programmed Cell Death 4 (PDCD4), Troponymosin-1 (alpha) (TPM1), Mammary Serine Protease Inhibitor (SERPINB5), and Reversion-Inducing-Cysteine-Richprotein with Kazal motifs (RECK). It has been shown that miR-21 has a crucial role in disrupting growth by inducing apoptosis. miR-21 had used to know the profile CP/CPPS and clear cell renal cell carcinoma therefore miRNA-21 also used as a potential marker to know PCa [5,6]. Programmed Cell Death 4 (PDCD-4) is known as a tumor suppressor gene. PDCD-4 decreased in common tumor entities. The Reduction of PDCD-4 expressions potential urinary biomarkers in PCa Programmed Cell Death 4 (PDCD-4) is a tumors upper organs that has been decreased in Regulation formany tumor entities. Increased regulation of PDCD-4 can be found after the initiation of apoptosis, contrary to the reduction of PDCD-4 expression can contribute the anti-apoptotic nature of cancer cells [7]. We aimed to investigate miR-21 and PDCD-4 expression as potential urinary biomarkers in PCa.

Material and Methods

Sample collection and exosome isolation

Urine samples collected from 145 patients were examined, 45 patients diagnosed with Benign Prostate Hyperplasia (BPH), and 100 patients diagnosed with PCa. All of the patients participated in this study signed written consent. This study received ethical approval from the Institutional Review Board of Universitas Gadjah Mada (approval number: KE/FK/0449/EC/2019). We collected 15 ml of urine from each patient. The samples were then distributed into four vials (1.5 mL), and each vial contained 1 mL of the urine sample. After the centrifugation, the supernatant was extracted and filled into new a vial and kept in a refrigerator at -80°C.

RNA isolation and cDNA synthesis

The total RNA was extracted using the miRCURY RNA Isolation
Kit-Bioflud kit (Exiqon, Denmark). Complementary DNA (cDNA) was synthesized using a Universal cDNA Synthesiskit (Exiqon, Denmark).

Quantitative polymerase chain reaction (qPCR) and data analysis

Quantitative PCR was conducted using an ExiLent SYBR Green Master Mixkit (Exiqon, Denmark), primer set (forward and reverse), and diluted cDNA. The qPCR analysis of gene expression was performed using the qPCRcEX96 thermocycler (Bio-Rad). All of the procedures followed the manufacturer’s recommendations, and statistical analyses were performed using the SPSS Version 23 and Graph Pad Prism 7. In this study, statistical significance was set at p-value <0.05 (Table 1 and 2).

Mean age of the samples by BPH, non-metastatic PCa and metastatic PCa were older than 65 years old. Characteristic median PSA were appropriate as predisposition lower in BPH rather than PCa.

The median age of the samples were 65(46-88), 69.5(52-84) and 67(49-82) for BPH, non-metastatic PCa, and metastatic PCa, respectively. The median PSA score for BPH, non-metastatic PCa, and metastatic PCa were 3.8(0.4-20.82), 37.3(2.51-386) and 67(49-82) for BPH, non-metastatic PCa, and metastatic PCa, respectively. The median PSA score for BPH, non-metastatic PCa, and metastatic PCa were appropriate as predisposition lower in BPH rather than PCa.

The expressions of each biomarker were analyzed using Mann Whitney Test. We found a significant difference between miR-21-5p expression in the BPH group and PCa, both metastatic and non-metastatic, withp-values of 0.017 and 0.004, respectively. Never theless, expression of miR-21-5p in metastatic and nonmetastastic PCa showed in significant results. BPH group showed the highest expression of PDCD-4 mRNA. Significant difference was found between BPH group and PCa group, both metastatic and non-metastatic.

In this study, miR-21-5p showed potential diagnostic value to detect PCa. The expression of miR-21-5p, using 1.34 as cut-off point had 83% sensitivity and 44.4% specificity.

Discussion

Uregulation of urine-based miR-21-5p was significantly higher in patients with PCa than patient with BPH. This study indicated that the overexpression of urine-based miR-21-5p could be a potential non-invasive biomarker for diagnostic aspects of PCa which was aligned with our previous study [8]. This result was supported by previous studies which stated that the overexpression of miR-21 in urine and serum samples of PCa patients played an essential role as a diagnostic and prognostic biomarker [9-13]. PCa proliferation and invasion were significantly decreased by the inhibition of miR-21-5p [14]. Studies conducted by Li T. et al. and Ghorbanmehr, et al. showed increased miR-21-5p expression in urine sample from PCa patients [15,16]. As one of the best studied miRNAs, miR-21-5p demonstrated its oncogenic activity in most cancers [17]. Additionally, several previous studies suggested that oncomiR miR-21 had been involved in many solid and haematological organ malignancies [18-23]. It has been reported that miR-21-5p was expressed higher in PCa tissue than in normal prostate tissue [24,25]. Melbo-Jorgensen C. et al. showed overexpression of 21-5p in prostate cancer patients undergoing radical prostatectomy [26]. Further more, miR-21-5p, which was shown to contribute to prostate cell transformation and had been associated with cancer initiation, progression, and metastasis [27-29], correlated with stronger PCa cell growth both in vitro and in vivo [30]. In addition, it provided resistance to docetaxelin PC-3 cells, although, knockdown of miR-21 in human cell was sensitive to docetaxel-induced apoptosis [31]. Same study by Kopczynska miRNA had a role in the resistance of prostate cancer with docetaxel and paclitaxel [32]. Study conducted by Porzycki et al. which showed the potential role of miR-21 as a potential diagnostic biomarker, had an analysis done using the ROC curve with the results of the area under the curve of 0.856 [10]. This findings was aligned with the study conducted by Stuopelyte et al. whose area under the curve value was 0.633. The urine specificity of miR-21 was higher than that of PSA (76.22% vs 63.57%) but the sensitivity value was almost the same as PSA (47.83% vs 52.38%)[33].Our study showed the similar result, where the value of the area under the curve on the ROC miR-21-5p curve is 0.658. Compared with the previous studies, the sensitivity of miR-21-5p in this study was found to be higher (79%) while the specificity was lower (44%).

In this study we found that the expression of PDCD-4 mRNA was lower in PC ag rouph than in BPH group. Lowe expression was found in metastatic group compared to non-metastatic group. This study indicated that lower expression of urine-based PDCD-4 could be a potential non-invasive biomarker for prognostic aspects of PCa. Several studies showed that the PDCD-4 mRNA was expressed lower in the PCa group compared to BPH. Lower expression was also found inmetastatic group compared to the non-metastatic group. Consistent with previous studies, a significant difference in the PDCD-4 expression was found between PCa and prostate hyperplasia [34]. Down regulation of the PDCD-4 gene in prostate cancer tissue was also found in a study by Fischer N. et al. In PDCD-4 nuclear and cytoplasmatic staining, there was a significant decrease in prostate cancer tissue [25]. Reduced PDCD-4 expression was associated with PCa progression and pathologic features [21,35,36]. Studies conducted in mice, proved that PDCD-4 was a true tumor suppressor [37,38]. A recent study by Aameri, observed localization of PDCD-4 mainly in nuclei epithelial cells in normal but in prostate cancer specimens [35]. Another study by Zennami, PDCD-4...
mRNA and protein level was significantly decreased in higher Gleason score tumor using 546 patient samples [39]. In a study conducted by Zennami et al. demonstrated that PDCD4 regulates proliferation, apoptosis and castration resistance in prostate cancer [40]. Matsuhashi et al. found that PDCD-4 protein synthesis was inhibited by miR-21 in prostate cancer. Dong et al. also found that PDCD-4 mRNA expression was decreased in prostate cancer compared to IL-6-inhibited BPH. In this study, it is known that PDCD4 expression in urine can be a diagnostic tool for prostate cancer with a sensitivity value of 90%, but with a specificity of 42.2%. Translation of tumor suppressor gene PDCD-4 is negatively regulated by miR-21(34) [41]. As the target of miR-21 regulation in PCa cells, PDCD-4 expression will be reduced by miR-21, and lower PDCD-4 expression correlates with tumor cell invasion and distant metastasis in PCa. Other studies showed androgen stimulation in PCa cell line associated with lower mRNA expression of PDCD-4 in non-metastatic compared to metastatic PCa group could be potential prognostic biomarker with a specificity of 42.2%. Translation of tumor suppressor gene PDCD-4 is negatively regulated by miR-21 [34] [41].

Conclusion

The overexpression of miR-21-5p shown in this study could be a potential non-invasive diagnostic tool for patients with PCa. The lower mRNA expression of PDCD-4 in non-metastatic compared to metastatic PCa group could be potential prognostic biomarker in PCa. Further studies with larger population are required to investigate the role of miR-21-5p and PDCD-4 as biomarkers in PCa. The combination of both urinary based miRNA and mRNA could investigate the role of miR-21-5p and PDCD-4 as biomarkers in PCa.

References


