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Original Research Article

Detection of miRNAs in urine of prostate cancer patients

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ABSTRACT

Background and aim: Prostate cancer (PCa) is the second most prevalent oncologic disease among men worldwide. Expression of various transcripts, including miRNAs, is markedly deregulated in cancerous prostate tissue. This study aimed at identifying a PCa-specific expression profile of miRNAs for subsequent use in noninvasive diagnostics.

Materials and methods: MiRNA expression was profiled in 13 PCa tissues using human miRNA microarrays. Highly expressed miRNAs were selected for the analysis in urine of patients with PCa ($N = 143$) and benign prostate hyperplasia (BPH; $N = 23$) by means of real time PCR, while miRNAs showing the expression differences in relation to clinical variables were further analyzed in 52 PCa and 12 noncancerous prostate tissues (NPT) on TaqMan Low Density Arrays (TLDA).

Results: Analysis of miRNA expression in prostate tissue linked miR-95 to aggressive form of PCa. This miRNA was up-regulated in high grade ($P = 0.041$), the *TMPRSS2-ERG* fusion-positive tumors ($P = 0.026$), and in patients with subsequently developed biochemical recurrence (BCR; $P = 0.054$) after radical prostatectomy. MiRNAs highly expressed in PCa tissues were also detectable in urine from PCa patients. Moreover, the urinary levels of miR-21 had significant discriminatory power ($P = 0.010$) to separate PCa patients from BPH, while the combined analysis of urinary miR-19a and miR-19b was prognostic for BCR. In PCa, the diagnostic potential of urinary miRNA panel (miR-21, miR-19a, and miR-19b) was higher than that of the PSA test (AUC = 0.738 vs. AUC = 0.514).

Conclusions: Measurement of urinary levels of PCa-specific miRNAs could assist in more specific detection of PCa and prediction of BCR.

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1. Introduction

Prostate cancer (PCa) is the second most prevalent male cancer worldwide and the predominant male cancer in developed countries [1]. While many prostate tumors grow slowly, are confined in the prostate and do not reduce the life quality of patients, others are aggressive, spread quickly and, therefore, should be detected at earlier stages. Despite its high false positive and false negative rates, the prostate-specific antigen (PSA) test remains the only widely available test for PCa screening and diagnostics [2]. Nowadays, studies of PCa are directed toward development of novel non-invasive tests that can improve the specificity of PSA testing and provide at least modest prognostic information about the disease aggressiveness. However, none of the novel PCa-specific molecular biomarkers is currently approved for routine clinical usage.

MicroRNAs (miRNAs) are single-stranded small (18–24 nt) non-coding RNAs that originate endogenously and function in the post-transcriptional regulation of gene expression. Target mRNA undergoes transcriptional repression or cleavage according to complementarity level of its 3′ UTR and the seed region (2–8 nucleotides) of miRNA [3]. In that way one single miRNA is able to have multiple targets as well as a single mRNA can be regulated by multiple miRNAs. Regulatory capabilities of miRNAs affect nearly all physiological processes such as cell growth, differentiation, and apoptosis. In contrary, many human diseases, including cancer, are associated with deregulation of miRNAs production [4]. In cancer research, in analogy with cancer-related genes, up-regulated and down-regulated miRNAs are called oncogenic miRNAs (onco-miRs) and tumor suppressor miRNAs, respectively [5]. In many forms of cancer, miRNA expression profile significantly differs between cancerous vs. non-cancerous tissues, between aggressive vs. indolent cases, and may change simultaneously with the disease progression [6]. MiRNAs are also detectable in cell-free form circulating in various body fluids [7]. During oncogenesis, the content of freely circulating miRNAs changes and can reflect the situation in tumor [8]. Circulating miRNAs are firmly packed and remain quite stable in body fluid even after collection [9], providing possibilities to serve as non-invasive tools for detection of disease-specific changes. Recent studies of circulating miRNAs in PCa [10,11] revealed their good diagnostic potential when used as a single biomarker or in combination with the PSA test [12]. The prevalent source to analyze circulating miRNAs in PCa is blood [12,13], whereas a quite limited number of studies has been done on more specific body fluids such as urine [11], seminal fluid [14] or ejaculate [15].

In the present study, miRNA profiling was performed in PCa tissues by miRNA microarrays, while expression levels of selected miRNAs were analyzed in PCa and noncancerous prostate tissues on custom designed TaqMan Low Density Arrays (TLDA). A subset of miRNAs was selected for the investigation in urine of PCa and benign prostatic hyperplasia (BPH) patients. Changes of miRNA levels in urine were disease-specific and correlated with the biochemical disease progression after surgery.

2. Materials and methods

2.1. Sample collection

Prostate specimens were obtained prospectively from PSA-screened and biopsy-proven intermediate-risk PCa patients treated with radical prostatectomy (RP). Surgery was performed at the Urology Department of Vilnius University Hospital Santariškių Klinikos from January 2008 to May 2011. The local Bioethics Committee approved the study before its initiation, and all patients gave informed consent for participation in the study.

The investigation was a part of the large-scale PCa biomarker study performed according to standardized protocols of sample collection and processing as reported previously [16,17]. Briefly, after RP, prostate tissue cores of 0.8 cm diameter were prepared from unfixed prostate and immediately frozen in liquid nitrogen. Before freezing, sections of tissue were placed on microscopic slides for quantification of tumor cells using hematoxylin and eosin staining. Cancerous (≥70% of cancer cells) and non-cancerous (0%) prostate tissues were sampled by expert histopathologist and transferred for molecular analysis. For the RNA extraction frozen tissue sections from 65 PCa patients were available, including 53 prostate adenocarcinomas and 12 noncancerous prostate tissues (NPT).

In addition, 166 samples of urine sediments were collected from 23 BPH and 143 PCa patients, 40 of which had a corresponding tissue sample. Urine was catheterized during RP, and 30 mL of collected urine were centrifuged within a half an hour at 1000 rpm for 15 min at 4 °C and PBS-washed three times, and stored at −70 °C until use.

Due to overlapping cases in tissue and urine analyses, the total number of PCa cases of our study was 156, with follow-up data available for 144 (92.3%) of them. Biochemical recurrence (BCR) was defined as an increase in PSA level to ≥0.2 ng/mL after RP. 34 (23.6%) of PCa patients experienced BCR in 22 months after surgery on average (range 0–64 months). The mean follow-up time of cases without progression was 36 months (range 4–66 months). Clinical–pathological characteristics are summarized in Table 1. The status of the fusion transcript *TMPRSS2-ERG* was identified in our previous study [16,18].

2.2. Total RNA purification from tissue

Total RNA from prostate tissue was isolated with mirVana™ miRNA Isolation Kit (Ambion, USA) according to manufacturer's protocol. About 30 mg of mechanically homogenized tissue were lysed with 600 μL Lysis/Binding Buffer and extracted total RNA was eluted in 100 μL of 95 °C temperature Elution Solution.

2.3. Total RNA purification from urine sediments

miRNeasy Mini Kit (Qiagen, USA) was used for RNA isolation from urine according to manufacturer's protocol, which adjusts kit for the purification of total RNA from liquid samples. Total RNA was extracted from 200 μL of urine