

Differential Expression of MicroRNAs Assayed from Urine of Patients with Prostate Cancer or BPH

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ABSTRACT

Prostate cancer is the most frequently occurring cancer and is the second highest cause of cancer mortality in males. Serum prostate specific antigen (PSA) is currently used as an indicator for the diagnosis and management of prostate cancer. Patients with a serum PSA level between 2.5 ng/mL and 10 ng/mL will often undergo prostate biopsies to confirm prostate cancer. However, <30% of these men will biopsy positive for cancer; meaning that the majority of men underwent an invasive biopsy with little benefit. A non-invasive biomarker, which can detect prostate cancer at an early stage, is therefore urgently needed.

MicroRNAs (miRNAs) are small endogenously expressed non-coding RNAs that primarily down-regulate expression of protein-coding genes at the translational level. Accumulating evidence, such as aberrant expression of miRNAs, suggests that they play a vital role in the development of cancer. They have been identified in various tumor types, demonstrating that different sets of miRNAs are usually deregulated in different cancers. Toward this, we investigated the differential expression profile of urinary miRNAs among patients with either Prostate Cancer or Benign Prostatic Hyperplasia as well as healthy control individuals.

MicroRNA expression profiles were generated by microarray. A number of miRNAs were found to be differentially expressed between prostate cancer and benign prostatic hyperplasia when compared against microRNA expression from the healthy control group. The differential expression of these miRNA candidates is being confirmed by evaluating the expression of targetted mRNAs known to be involved in carcinogenesis. These miRNAs, if valid might be of significant use as novel biomarkers towards the early detection of prostate cancer.

INTRODUCTION

Prostate Cancer is the leading diagnosed cancer of Canadian men with 25,500 cases diagnosed in 2011. Mortality for prostate cancer makes up 10% of cancer related deaths in Canadian men with 4,100 deaths due to the disease in 2011.

The current PSA blood test for prostate cancer, with a sensitivity of 86% and a specificity of 33% at a cut-off of 4.0ng/mL is very unreliable and results in many false positives and therefore unnecessary, painful and costly biopsies. At least one of the two large scale studies (over 75,000 and 180,000 participants), both published in the New England Journal of Medicine in 2009, suggests that even yearly screening of total PSA had no significant affect on prostate cancer mortality.

Urine has been shown to be a good sample source for miRNA as it is obtainable non-invasively, in large quantities and with little training; it is also non-infectious for many pathogens including HIV.

MicroRNAs play an important role in the development and progression of cancer due to their ability to regulate genes post-transcriptionally. Differential expression of miRNAs has been linked to various cancers including prostate cancer.

MATERIALS & METHODS

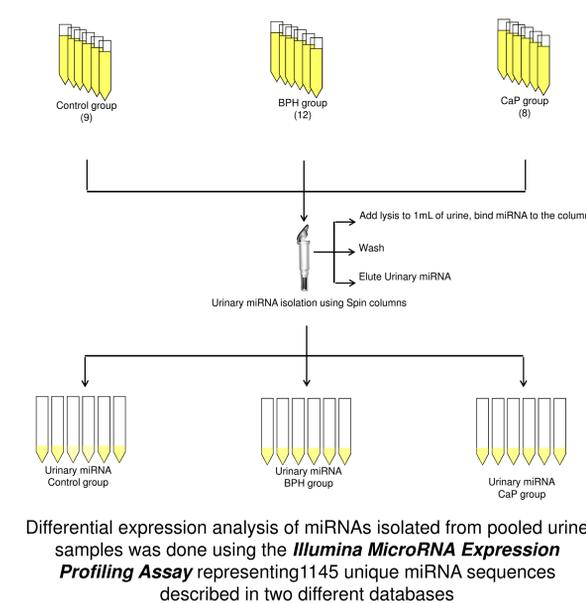


Figure 1. Methods for isolating & detecting differential expression of urinary miRNAs. Flow diagram demonstrating the isolation of miRNA from urine using Norgen's Urine miRNA Isolation Kit followed by analysis of differential expression using Illumina's miRNA expression Profiling Assay.

RESULTS

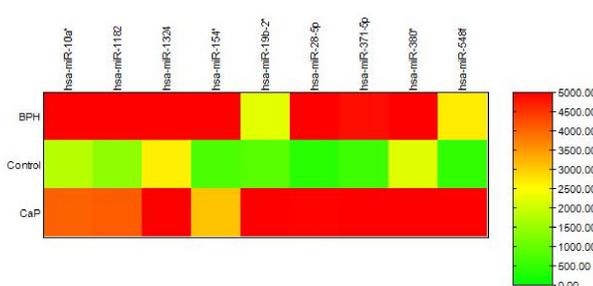


Figure 2. Commonly Over-Expressed miRNAs in both BPH and CaP. Urinary miRNAs were isolated from the urine of 9 healthy individuals, 8 Prostate Cancer (CaP) patients and 12 patients with Benign Prostatic Hyperplasia (BPH). Urinary miRNAs from each group were pooled and assessed for differential expression between the three groups. Nine miRNAs were found to be commonly over-expressed among CaP and BPH samples with respect to the healthy control group.

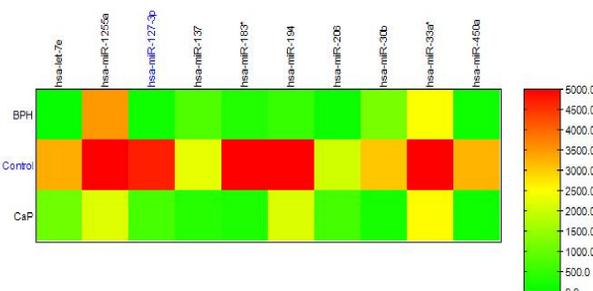


Figure 3. Commonly Suppressed miRNAs in both BPH and CaP. Urinary miRNAs were isolated from the urine of 9 healthy individuals, 8 Prostate Cancer (CaP) patients and 12 patients with Benign Prostatic Hyperplasia (BPH). Urinary miRNAs from each group were pooled and assessed for differential expression between the three groups. Ten miRNAs were found to be commonly suppressed among CaP and BPH samples with respect to the healthy control group.

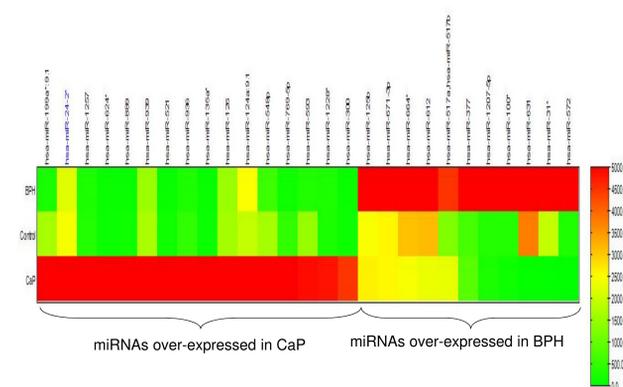


Figure 4. MicroRNAs that were over-expressed solely in the BPH or CaP samples. Urinary miRNAs were isolated from the urine of 9 healthy individuals, 8 Prostate Cancer (CaP) patients and 12 patients with Benign Prostatic Hyperplasia (BPH). Urinary miRNAs from each group were pooled and assessed for differential expression between the three groups. Sixteen miRNAs were found to be over-expressed only in CaP samples; while eleven miRNAs were found to be over-expressed only in BPH samples.

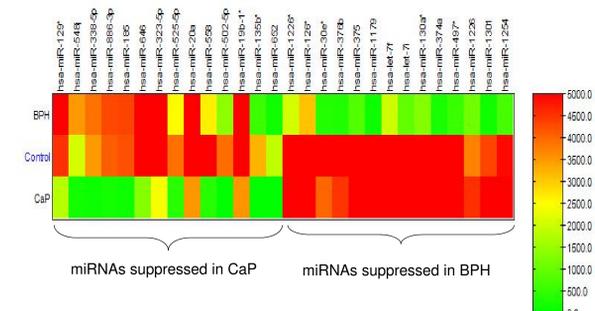


Figure 5. MicroRNAs that were under-expressed solely in the BPH or CaP samples. Urinary miRNAs were isolated from the urine of 9 healthy individuals, 8 Prostate Cancer (CaP) patients and 12 patients with Benign Prostatic Hyperplasia (BPH). Urinary miRNAs from each group were pooled and assessed for differential expression between the three groups. Fourteen miRNAs were found to be under-expressed only in CaP samples while another fourteen miRNAs were found to be suppressed only in BPH samples.

CONCLUSIONS

- Urine is a viable source of microRNA. Despite the low concentration, small non-degraded RNA species can be isolated when the appropriate method is employed. The RNA is of sufficient quality for expression profiling to generate meaningful data that reflects what is happening in the prostate.

- A number of microRNAs were found to be differentially expressed among the three pooled groups of samples. Several of them agree with previous data found in the literature for known species of microRNA that are either commonly, up or down regulated in various cancers, prostate cancer among them.

- The differential expression of these biomarker candidates will be confirmed by evaluating the expression of cancer related messenger RNAs targeted by these specific miRNAs. These miRNAs, if valid might be of significant use as novel biomarkers towards the early detection of prostate cancer.