Abstract

The two studies follow free nucleic acids in urine in search for biomarkers to distinguish urinary bladder cancer patients from controls. Bladder cancer forms 4 % of newly diagnosed oncological diseases in the Czech Republic. Nowadays, there is no accredited non-invasive method for its diagnosis, which is sufficiently accurate. Urine supernatant, which is washing the bladder mucosa and which does not contain cell debris, seems to be an appropriate source of biomarkers for non-invasive diagnosis.

miRNAs, as a non-invasive biomarker of urinary bladder cancer, were studied in one of the studies. miRNAs are short noncoding RNA, which block the process of translation. miRNAs occur in all body fluids and are relatively stable.

A study with three phases was assessed to find a suitable miRNA marker. 109 individuals were examined in total (36 controls and 73 bladder cancer patients). The analysis of miRNAs was based on RT-PCR (Reverse Transcription Polymerase Chain Reaction). In the first phase, the urine of 59 individuals was analyzed on TaqMan array card with 381 miRNAs. In the second phase, the results of the first phase were confirmed on the same cohort by a single miRNA assay. In the third phase, a new cohort was used (23 controls and 27 bladder cancer patients), analyzed by a single miRNA assay again. All the results were normalized to three miRNAs, which were chosen by geNorm algorithm within the qBase$^{+©}$ program: miR-191, miR-28-3p, and miR-200b.

Five miRNAs were found to be down-regulated in the urine supernatant of bladder cancer patients: miR-125b, miR-30b, miR-204, miR 99a, and miR-532-3p. The best results were reached with miR-125b and miR 99a.

These results show that miRNA levels can be used as a diagnostic marker for non-invasive detection of bladder cancer.

Levels of cell-free DNA (cfDNA) as a biomarker for urinary bladder cancer were measured in the second study. Altogether, 100 individuals were examined (34 controls and 66 bladder cancer patients). The volume of each portion of voided urine was recorded, and cfDNA concentration was measured by real-time PCR. This way the total

amount of cfDNA was calculated. The second portion of morning urine was assessed as the better one for the calculation of cfDNA total amount than the first morning portion.

The methodology for measuring the cfDNA in urine was proposed. Calculation of the total cfDNA amount in the second morning urine is capable of distinguishing bladder cancer patients from controls ($p=0.0002$).

When following correct methodological procedures, quantification of cfDNA in urine supernatant has the potential to serve as a non-invasive diagnostic marker for bladder cancer.

Key words: miRNA, cfDNA, urine, bladder cancer, non-invasive marker