Introduction
Prostate cancer (PCa) is the second most prevalent malignancy of males characterized by high mortality rates. PCa can be effectively treated if it is diagnosed in its early stages. Recently, DNA methylation has been proposed as one of the most important events in prostate carcinogenesis. It occurs at early stages of tumor development and can be detected by noninvasive means, including analysis of urine sediments from PCa patients.

Aim
In order to evaluate the suitability of epigenetic biomarkers for early detection of PCa, we analyzed promoter methylation changes of genes RARB, RASSF1, and GSTP1 in PCa and benign prostatic hyperplasia (BPH). To determine the clinical utility of noninvasive PCa detection we analyzed urine samples from the same PCa and BPH patients.

Patients and methods
In our study quantitative methylation-sensitive PCR (QMSP) was used for detecting aberrant promoter methylation in 102 samples of urine sediment from previously untreated cases of biopsy-proven early (pT2, n=84) or medium stage (pT3, n=16) PCa of grade 6 (n=71) or 7 (n=31) according to Gleason system and 5 cases of BPH (Fig. 1).

The level of promoter methylation for particular gene was evaluated by calculating percentage of methylated reference (PMR) using ACTB as endogenous control.

Aberrant promoter methylation was also analyzed in 32 PCa and 19 BPH tissues by means of methylation-specific PCR (MSP) (Fig. 2).

Results
- Hypermethylation of all analyzed genes was detected both in prostate tissues and urine sediment samples (Fig. 3).
- In PCa tissues aberrant methylation of each of the genes correlated with tumor size (P<0.05). Significant associations were also observed between RASSF1 hypermethylation and Gleason grade (P<0.01) or PSA concentration (P=0.03).
- At least one of the three genes was hypermethylated in urine sediments in 68 of 102 PCa cases (67%), and 14 of 102 (14%) samples were positive for hypermethylation of at least two genes (Fig. 4).
- The average PMR for positive cases was 58%, 11%, and 11% for RASSF1, RARB, and GSTP1, respectively.
- High level of methylation (PMR≥50%) was detected in 36 of 102 (35%) cases for RASSF1, while PMR value for RARB and GSTP1 reached only 31% and 17%, respectively.
- Hypermethylation of RASSF1 at relatively lower level was also detected in urine sediments from BPH patients (average PMR was 20%).
- In urine sediment samples from PCa patients PMR level for GSTP1 methylation correlated with prostate weight (P=0.015).

Conclusions
Preliminary results of our study show high sensitivity and specificity of particular DNA methylation biomarkers for early and noninvasive detection of prostate cancer.

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