DNA methylation biomarkers for noninvasive detection of prostate cancer

Kristina Daniunaite[1], Feliksas Jankevicius[2,3], Juozas Lazutka[1], and Sonata Jarmalaite[1]

[1]Faculty of Nature Sci., Vilnius University; [2]Faculty of Medicine, Vilnius University, [3]Vilnius University Hospital Santariskiu Clinics, Lithuania

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).

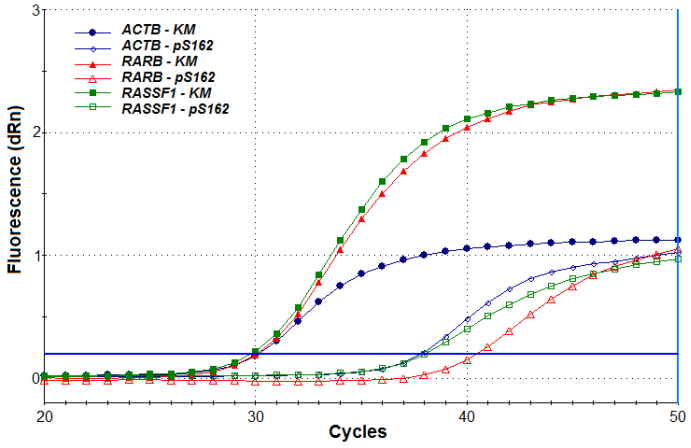

Fig. 1. Amplification plots of QMSP assay of a particular urine sample (pS162). *RARB* and *RASSF1* – genes of interest, *ACTB* – reference gene used to normalize for DNA input, KM – *in vitro* fully methylated leukocyte DNA. Baseline-subtracted fluorescence signal is normalized to the passive reference dye ROX and expressed in relative units.

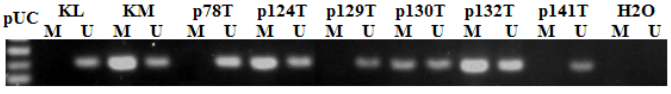

Fig. 2. MSP analysis of gene *RARB* in PCa tissue samples. KL – leukocyte DNA from healthy donor, KM - *in vitro* methylated leukocyte DNA, p78T-p141T – PCa samples, H2O – non-template control, pUC – DNA size marker, M – methylated DNA, U – unmethylated DNA.

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).

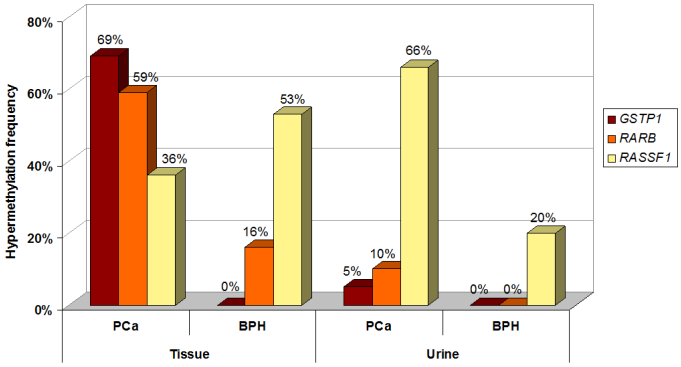

Fig. 3. Hypermethylation frequencies of genes *GSTP1*, *RARB*, and *RASSF1* in prostate tissues and urine sediment samples. Hypermethylation analysis in BPH tissues was done by N. Osipova.

- 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.

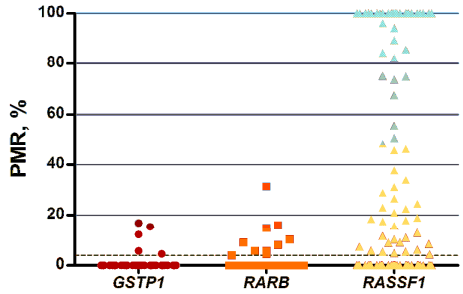

Fig. 4. PMR values for *GSTP1, RARB, and RASSF1* in urine sediments from patients with diagnosed PCa. Dashed line – cut-off at 4%.

- 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.

Acknowledgements

The study was supported by grant C–03/2007-09 from Lithuanian State Science and Studies Foundation, grant MIP 109 from the Research Council of Lithuania. Kristina Daniunaite acknowledges Student Research Fellowship Award from the Lithuanian Science Council.