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Frequent Methylation of *RASSF1* and *RARB* in Urine Sediments From Patients with Early Stage Prostate Cancer

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***Key words**: prostate cancer; DNA methylation; urine sediments; RASSF1 gene, RARB gene.*

***Summary.** Background. Prostate cancer (PCa) is the second most prevalent malignancy among males, characterized by high mortality rates. Aberrant DNA methylation in promoters of tumor suppressor genes is an early and frequent event during prostate carcinogenesis. Modern techniques allow a sensitive detection of DNA methylation biomarkers in bodily fluids from cancer patients offering a noninvasive tool for PCa monitoring. Our study aimed at the analysis of DNA methylation in urine sediments from PCa patients for the selection of most informative noninvasive biomarkers.*

Material and Methods. Real-time methylation-specific polymerase chain reaction was used for the detection of methylated RASSF1, RARB, and GSTP1 genes in catheterized urine specimens from 34 patients with biopsy-proven early or medium stage PCa.

Results. At least one gene was methylated in urine sediments from 28 cases with PCa, with a sensitivity of the test reaching 82%. RASSF1 was methylated in 71% (24 of 34), RARB in 44% (15 of 34), and GSTP1 in 3% (1 of 34) of the specimens. High level of methylation (⩾50%) in RARB and RASSF1 genes was detected in 40% and 20% of cases, respectively. A significant association was observed between high level of RARB methylation and Gleason score (P=0.01), while methylation of at least one gene occurred more frequently in urine DNA of older patients (P=0.02).

Conclusions. Results of our study show a high sensitivity of DNA methylation biomarkers, especially RASSF1 and RARB, for the early and noninvasive detection of PCa.

Introduction

Prostate cancer (PCa) is the second most common cancer and the fifth leading cause of death from cancer among men worldwide (1). In 2009, more than 3000 new cases of PCa were diagnosed in Lithuania. In our country, it is the most prevalent cancer and the second leading cause of death among men (2). PCa can be effectively treated if it is diagnosed in its early stages, when the tumor is still confined to the prostate. Currently, prostate-specific antigen (PSA) is the only molecular biomarker routinely used for PCa detection and monitoring of disease recurrence (3). When the level of PSA is increased, prostate biopsy is recommended, with 4 ng/mL of PSA being the usual threshold level. However, this protein is specific for prostate tissue, but not for PCa. Such noncancerous conditions as benign prostatic hyperplasia (BPH), prostatitis, or prostatic ischemia can cause the elevated levels of PSA (1, 4). The low specificity of PSA leads to a high number of unnecessary biopsies, causing avoidable patient discomfort. Therefore, the development of a minimally invasive, yet specific, tool to aid in the early detection of PCa is needed.

Cancer arises through the accumulation of multiple molecular events, which include changes in gene expression through epigenetic mechanisms (5). Initially found in DNA isolated from tissues of various tumors, epigenetic alterations, especially hypermethylation in the promoter regions of tumor suppressor genes, have been increasingly demonstrated in bodily fluids (blood serum or plasma, urine, ejaculate, sputum) from cancer patients (6). DNA methylation biomarkers, unlike RNA and protein alterations, are relatively stable in bodily fluids and occur in well-defined regions, unlike DNA mutations (3), thus can serve as a simple and sensitive noninvasive tool for the early detection of cancer and further monitoring of disease outcome. For routine application of DNA methylation biomarkers in clinical practice, the most informative set of genes should be selected for every particular type of cancer and the most effective methods of analysis should be developed for the sensitive detection of minor amounts of tumor-derived cells or cell-free DNA circulating in bodily fluids.

Recent studies of DNA methylation in prostate

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tumors have revealed a panel of genes frequently hypermethylated in PCa (3, 5). The most common epigenetic alteration in prostate carcinogenesis is hypermethylation in the promoter region of the glutathione-S-transferase P1 (*GSTP1*) gene that leads to decreased expression of an intracellular detoxification enzyme. *GSTP1* promoter methylation is present in up to 90% of prostate cancer tissues and two-thirds of intraepithelial neoplasia tissues but rarely is present in BPH tissue (7). Methylation of *GSTP1* is also detectable, but with the lower frequencies, in plasma, urine, and ejaculates of PCa patients (7–16). Other genes that are commonly found methylated in prostate cancer include *RASSF1, RARB, APC*, *MDR1*, *PTGS2*, *TIMP3*, and *CDH1* (3, 5). Most investigations of epigenetic changes in PCa have mainly focused on the assessment of prostate tissue, while only a limited number of studies (9, 15–18) have analyzed a panel of DNA methylation biomarkers in urine sediments from PCa.

In urine specimens from 34 cases with biopsy-proven prostate adenocarcinoma, a panel of most commonly methylated genes in PCa that are associated with various cellular processes, particularly, *GSTP1* (glutathione S-transferase pi 1; detoxification of carcinogens), *RARB* (retinoic acid receptor beta; transcription regulation), and *RASSF1* (Ras association (RalGDS/AF-6) domain family member 1; cell cycle regulation), were analyzed. Real-time (quantitative) methylation-specific polymerase chain reaction (QMSP) was used for the sensitive detection of methylated DNA in catheterized urine specimens. Associations between aberrant promoter methylation of these genes and patients' demographic as well as clinical characteristics were examined. In addition, for comparison, the data on DNA methylation status of the analyzed genes in tumor tissues was available for the same set of cases from our previous study that showed high methylation frequencies of genes *GSTP1*, *RARB*, and *RASSF1* by means of methylation-specific polymerase chain reaction (MSP) (Daniūnaitė et al., unpublished data). The main aim of our study was to determine the sensitivity and clinical utility of this novel non-invasive test based on the detection of methylated DNA in catheterized urine of cancer patients.

Materials and Methods

Patients and Samples. Urine sediments were collected from 34 previously untreated cases who were enrolled into the study of PCa biomarkers (LSSSF project No. C03/2007) under the approved protocol for biopsy-proven prostate adenocarcinoma. Biosamples were collected from January 2008 to August 2009 in the Department Urology, Vilnius University Hospital Santariškių Klinikos, after the approval of study protocol by the local Bioethics Committee, and all patients gave informed consent for participation in the study. Tumor and nonmalignant prostate tissues were also available from most of the patients involved in this study.

The cases were diagnosed with early- (pT2, n=21) or medium-stage (pT3, n=13) prostate carcinoma of score 6 (n=24) or 7 (n=10) according to the Gleason system. No cases had pelvic lymph node involvement or clinical information of distant metastases. The mean age of the patients was 63 years (range, 52–77 years).

Thirty milliliters of urine was collected into urine collection cups by catheterization during radical prostatectomy. Biosamples were centrifuged at 1000 rpm for 15 minutes at 4°C and stored at –70°C before DNA extraction.

DNA Extraction and Quantitative Detection of DNA Methylation. Urine sediments (2 mL) were centrifuged at 1500*g* for 20 minutes and washed with 500 μL of PBS twice. The supernatant was decanted, and DNA was extracted by digestion with proteinase K, followed by a standard phenol-chloroform purification and ethanol precipitation. Sodium bisulfite conversion of unmethylated (but not methylated) cytosine residues to uracil was performed as described previously (19). Briefly, 720 ng of genomic DNA was denatured with 3 M NaOH for 15 minutes at 37°C and then exposed to bisulfite modification with 2.3 M sodium bisulfite (pH 5.0) and 10 mM hydroquinone at 50°C for 16 h (all reagents from Sigma-Aldrich Inc., Gillingham, Dorsel, UK). Modified DNA was purified with the Wizard DNA Clean-up System (Promega, Madison, WI) and desulfonated with 3 M NaOH, and then precipitated with ethanol.

The bisulfite-modified DNA was then used as a template for QMSP. For the *RARB*, *GSTP1*, and *RASSF1* genes, primers and hydrolysis probes (biomers.net, Ulm Donau, Germany) were designed according to published sequences (20, 21) to specifically amplify bisulfite-converted fully methylated DNA. A passive reference dye 6-carboxy-X-rhodamine (ROX) (Fermentas, Vilnius, Lithuania) was used for the normalization of fluorescence signal, in order to compensate for non–PCR-related variations in fluorescence. To normalize for DNA input in each sample, a myogenic differentiation gene 1 (*MYOD1*; primers from [21]) was used as a reference.

Fluorescence-based real-time PCR assays were carried out in a reaction volume of 20 μL consisting of 300 nM of each primer, 50 nM of probe, 1x Maxima Probe qPCR Master Mix (Fermentas, Vilnius, Lithuania), 30 nM of ROX, and 1 μL of bisulfite-converted DNA. PCR was performed in separate wells for each primer/probe set. Each sample was run in duplicate. Leukocyte DNA collected from healthy individuals was methylated in vitro using bacterial SssI methylase (New England BioLabs

Inc., Beverly, MA) and was included in each assay as a positive control (melthylated control, MC). Additionally, multiple nontemplate controls (NTCs) were included in each assay. All amplifications were carried out in 8-well strips (Applied Biosystems, Piscataway, NJ) on a Mx3005P system (Agilent Technologies Inc., Santa Clara, CA) under the following conditions: 95°C for 10 minutes followed by 50 cycles of 95°C for 30 s and 60°C for 1 minute. Fluorescence data were collected at the end of each 60°C step.

A run was considered valid when routinely included MCs gave a positive signal and NTC controls were not amplified. A urine sample was classified as valid if the quantification cycle (Cq) of *MYOD1* did not exceed 40, and amplification was detected in all replicates for a particular gene. The results were generated using MxPro v4.0 software (Agilent Technologies Inc., Santa Clara, CA) (Fig. 1).

Amplification-based threshold was used to determine Cq values. The percentage of methylated reference DNA (PMR) for each locus was calculated by dividing the *GENE*:*MYOD1* ratio of a sample by the *GENE*:*MYOD1* ratio of fully methylated control DNA and multiplying by 100. In this study, a sample was considered positive for methylation for a specific gene if PMR was >0 and there was no amplification in NTC controls.

Statistical Analysis. The two-sided Fisher exact test was used for comparison of categorical variables. The difference was considered significant if *P* value was <0.05. Odds ratio (OR) and Mantel-Haenszel 95% confidence intervals (CI) for two binomial samples were calculated using the approximation of Woolf. PMR value of each methylated sample was estimated automatically by the MxPro v4.0 software.

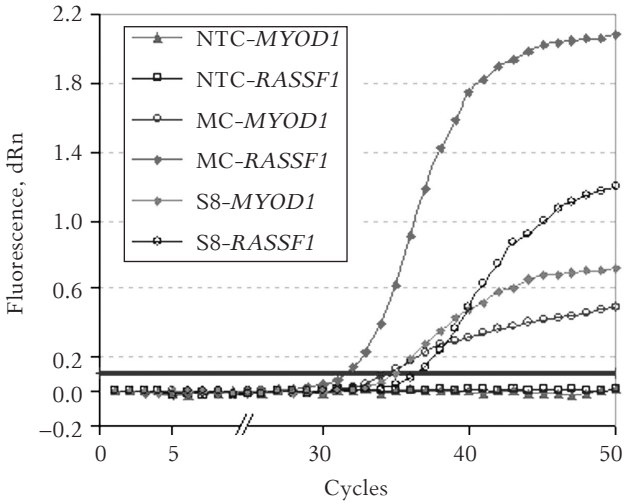

Fig. 1. Amplification plots of real-time methylation-specific polymerase chain reaction assay, generated with the MxPro v4.0 software

RASSF1, gene of interest; *MYOD1*, reference gene used to normalize for DNA input; NTC, nontemplate control; MC, in vitro fully methylated leukocyte DNA used as a positive control; S8, sample of urine sediments; horizontal solid line, amplification-based threshold. Baseline-subtracted fluorescence signal is normalized to the passive reference dye ROX and expressed in relative units.

Results

Of the 34 samples of urine sediments from PCa patients, at least one gene of the three genes (RARB,GSTP1, and RASSF1) included in the analysis was methylated in 28 cases (sensitivity of detection 82%), and 12 (35%) were positive for methylation of at least two genes. The frequencies of aberrant promoter methylation for *RASSF1*, *RARB*, and *GSTP1* were 71% (24 of 34), 44% (15 of 34), and 3% (1 of 34), respectively. PMR values ranged from 0.62% to 100% for *RASSF1* and from 6.35% to 100% for *RARB* (Fig. 2). High level of methylation (PMR≥50%) was found in 6 (40%) of the 15 cases for *RARB* and in 5 (20%) of the 25 cases for *RASSF1*. The only case with aberrant methylation for *GSTP1* was 100% hypermethylated.

In further analysis, gene methylation status in urine sediments was compared to DNA methylation

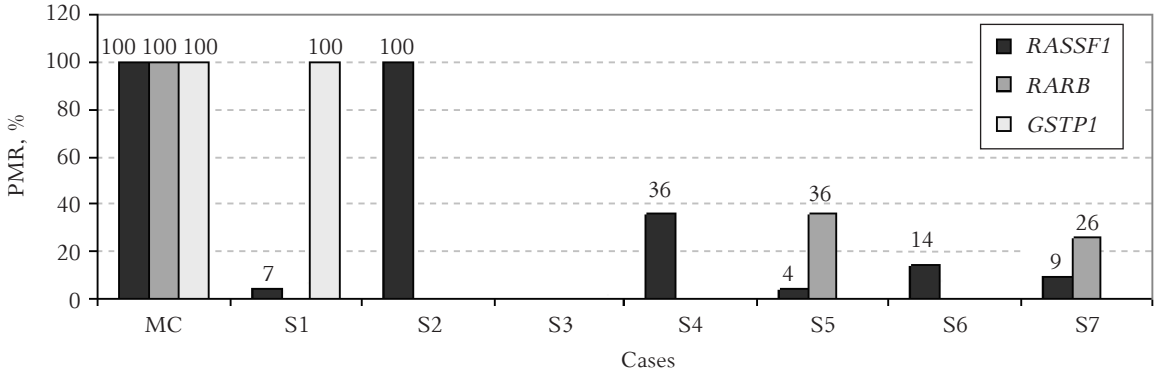

Fig. 2. Representative results of PMR values for *RASSF1*, *RARB*, and *GSTP1* in urine sediments from patients with diagnosed prostate cancer

MC, in vitro fully methylated leukocyte DNA used as a positive control; S1–S7, samples of urine sediments. DNA was partly methylated in samples S1, S4–S7 for *RASSF1* and samples S5 and S7 for *RARB*. Samples S1 and S2 were fully methylated for *GSTP1* and *RASSF1*, respectively, while no methylation for any of the genes was detected in sample S3.

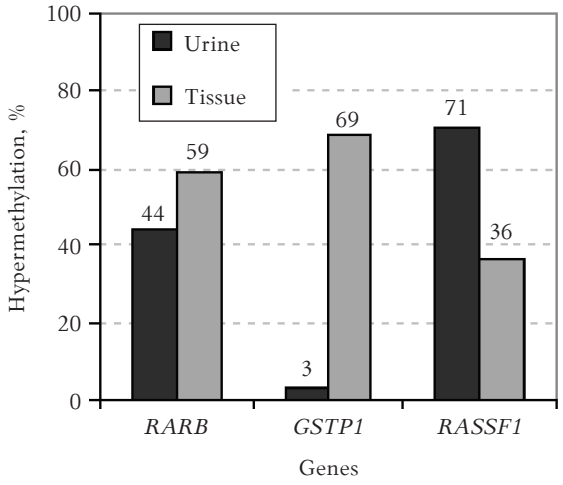

Fig. 3. Comparison of hypermethylation frequencies of genes *RARB*, *GSTP1*, and *RASSF1* in urine sediments and prostate carcinomas

status in tumor tissue from the same patient (Fig. 3). For such comparison, DNA methylation data collected by means of methylation-specific PCR were available for 32 prostate carcinomas (Daniūnaitė et al., unpublished data). Hypermethylation of *RARB* and *GSTP1* was more common in carcinomas than urine sediments, while *RASSF1* was more frequently methylated in urine sediments (Fig. 3). However, all the differences for the individual genes were statistically insignificant.

For 28 (88%) of the 32 patients, at least one gene with an identical methylation status in prostate carcinoma and urine was documented. When a gene was found to be methylated in prostate carcinoma, it was also methylated in urine sediments in 47% of cases for *RARB*, 100% of cases for *RASSF1*, and 5% of cases for *GSTP1*. For *RASSF1* and *RARB*, in some cases (47% and 13%, respectively), a gene was found to be methylated in urine but not in carcinoma tissue.

Next, we compared hypermethylation frequencies in urine samples according to demographic and clinical characteristics. Methylated *RARB* gene was more frequently detected in urine sediments from patients with higher prostate weight and in tumors of greater Gleason score; however, only quantitative measures of methylation showed statistically significant associations. High level of methylation (PMR$\geqslant$50%) of *RARB* was significantly more common in patients with score 7 prostate tumors as compared to score 6 (5/6 and 1/9, respectively; P=0.01; OR, 40.0; 95% CI, 2.0–794.9). There were no other significant associations between promoter hypermethylation of a particular gene and patient's age, tumor size or stage, Gleason score, preoperative serum PSA level, and prostate weight (Table). However, hypermethylation of at least one gene was more frequently observed in patients aged >63 years than patients aged $\leqslant$63 years (18/18 and 11/16, respectively; P=0.02; OR, 17.7; 95% CI, 0.9–351.2).

Discussion

Examination of epigenetic alterations in prostate cancer tissues has previously identified several potential biomarkers; however, for routine clinical application, noninvasive tests are preferable. Our pilot study in a set of 34 cases with early or medium stage prostate adenocarcinoma showed a high sensitivity (82%) of a panel of three DNA methylation markers (*RARB*, *GSTP1*, and *RASSF1*) for noninvasive detection of PCa using DNA from catheterized urine specimens. However, for further validation of diagnostic utility and specificity of these biomarkers, the assessment of DNA methylation changes in age-matched control group is of primarily importance.

Hypermethylation of *GSTP1* is an epigenetic biomarker most extensively studied in PCa. This alter-

Table. Aberrant Promoter Methylation in Urine Sediments From Prostate Cancer Patients According to Clinical and Demographic Characteristics

Characteristic		*RASSF1*		*RARB*		*GSTP1*	
		%	n/N	%	n/N	%	n/N
Age	$\leqslant$63 years	63	10/16	38	6/16	0	0/16
	>63 years	83	15/18	50	9/18	6	1/18
Stage	T2	81	17/21	48	10/21	5	1/21
	T3	62	8/13	38	5/13	0	0/13
Gleason score	6	75	18/24	38	9/24	4	1/24
	7	70	7/10	60	6/10	0	0/10
PSA*	<8 ng/mL	73	16/22	41	9/22	5	1/22
	$\geqslant$8 ng/mL	73	8/11	31	5/11	0	0/11
Tumor size	<12 cm^3	80	16/20	45	9/20	0	0/20
	$\geqslant$12 cm^3	64	9/14	43	6/14	7	1/14
Weight of prostate**	<62 g	68	15/22	32	7/22	5	1/22
	$\geqslant$62 g	80	8/10	60	6/10	0	0/10

PSA, prostate-specific antigen; N, number of analyzed cases; n, number of cases with methylated gene. *No data for one case; **no data for two cases. The mean value was used as a cut-off to stratify continuous variables into discrete values.

ation has been detected in around 90% of malignant prostate tissues and, in a lower frequency, in bodily fluids from PCa patients (4, 5). Suh et al. were ones of those who first reported the presence of methylated *GSTP1* promoter in ejaculates from 4 of the 9 patients with PCa (11). Later, other studies reported the presence of methylated *GSTP1* in urine samples from patients with PCa (7, 12). Including the most recent studies, hypermethylation frequency of *GSTP1* in urine sediments ranges from 18% to 83% (8, 10, 12–14, 16, 18). In our study, hypermethylation frequency of *GSTP1* was only 3%, which was lower than frequencies indicated in previous studies. The discrepancy could be explained by the different methods used in various studies to obtain urine sediment samples and to detect DNA methylation. It has been noted before that prostate massage could increase the shedding of prostate cells and, thus, the amount of methylated DNA in urine (10, 16), while in our study, the samples were collected by catheterization during prostatectomy aiming to avoid additional procedures for the patients. Moreover, Jeronimo et al. (10) reported that conventional MSP was more sensitive than real-time PCR in the detection of *GSTP1* hypermethylation in bodily fluids. In agreement with this notion, our assessment of DNA methylation by means of MSP (Fig. 3) rendered quite high *GSTP1* methylation frequencies in prostate adenocarcinomas. In addition, rare detection of methylated *GSTP1* in urine sediments can be impacted by predominance (69%) of the cases with early stage PCa in our study. The increased detection of *GSTP1* methylation in bodily fluids from patients with PCa of pronounced aggressiveness was shown in several studies (14, 22).

Although the reported frequencies of *GSTP1* methylation in urine sediments from PCa patients are comparably high, the combined analysis of several genes has a clear potential to increase the sensitivity of the test and minimize the false-positive rate. In urine sediments from PCa, the sensitivity of four-gene panel was 86%–87% in two studies (9, 16) that used real-time PCR for DNA methylation detection, while the large panel of 9 or 10 genes (9, 16) had a sensitivity of almost 100%. In our cohort of PCa cases, the combined sensitivity of 3 genes was 82%, and the most informative biomarkers of PCa were hypermethylated promoters of *RASSF1* and *RARB*. Up to date, only three previous studies have included the *RASSF1* and *RARB* genes in a panel of biomarkers analyzed in urine from PCa patients (9, 16, 17). The hypermethylation frequencies reported in these studies were 73%–78% and 35%–73% for *RASSF1* and *RARB,* respectively (9, 16, 17). In present study, *RASSF1* was determined to be most commonly methylated in our gene panel, with a frequency of 71%. The hypermethylation frequency of *RARB* (44%) also fits into the reported range. In agreement with other studies (7, 9, 12), our study demonstrated an association between DNA methylation status in urine sediments and tumor. In 88% of the patients, at least one gene with an identical methylation status in prostate carcinoma and urine was observed, confirming the origin of methylated DNA in urine from exfoliated tumor cells. However, in some cases, a gene was methylated in urine but not in carcinoma. It could be explained by technical differences in DNA methylation detection by MSP and QMSP assays, but mainly is attributable to multifocality of prostate cancer.

Several studies have shown not only a diagnostic but also prognostic potential of noninvasive DNA methylation tests. Associations have been detected between hypermethylation of several gene promoters in noninvasive specimens (serum, urine) from PCa patients and Gleason score, stage, or PSA value (14, 15, 23). Moreover, the presence of *GSTP1* hypermethylation in serum DNA from PCa patients was shown to be a significant predictor of PSA recurrence (22). In our study, high level ($\geqslant$50%) of *RARB* hypermethylation was significantly more common in urine from patients with PCa of greater Gleason score. Similarly, the quantitative measurement of DNA methylation in cancerous tissue revealed significant associations between increasing levels of methylation and advanced stage or greater score of PCa (24). In concern with previous data (17), our study detected more frequent DNA methylation in urine from older patients in support of the notion that aberrant methylation may accumulate during aging. Indeed, the age-related increase of DNA methylation levels in promoter region of the *GSTP1*, *RASSF1*, and *RARB* genes was shown in normal prostate tissues (25); however, age-matched prostate tumors contained significantly higher levels of methylation. Further analysis in urine samples from PCa patients and age-matched controls are of high importance to determine the aging-related and cancer-related levels of DNA methylation.

Conclusions

Results of our pilot study suggest that aberrant promoter methylation can be detected with high sensitivity in catheterized urine specimens from patients at early PCa stages. In addition, it shows that the panel of most informative genes may differ in various populations. In our cohort of patients with localized prostate cancer, *RASSF1* and *RARB*, but not *GSTP1*, genes are the most sensitive biomarkers of PCa. We continue our analysis in a larger set of cases and controls to evaluate the specificity of DNA methylation biomarkers for PCa. After further validation of these biomarkers in larger-scale studies involving the patients from different study centers, the detection of aberrant methylation in urine DNA may become a promising tool for the noninvasive

detection of prostate cancer, discrimination of cancer cases from benign conditions, monitoring for relapse, and measurement of therapeutic response.

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Statement of Conflict of Interest
The authors state no conflict of interest.

Dažnas genų *RASSF1* ir *RARB* metilinimas ankstyvąja priešinės liaukos vėžio stadija sergančiųjų šlapimo nuosėdose

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Raktažodžiai: priešinės liaukos vėžys, DNR metilinimas, šlapimo nuosėdos, *RASSF1*, *RARB*.

Santrauka. Priešinės liaukos vėžys yra antra pagal dažnumą vyrų onkologinė liga, kuriai būdingas didelis mirtingumas. DNR metilinimo pokyčiai naviką slopinamųjų genų promotoriuose dažnai nustatomi piktybėjančiuose priešinės liaukos audiniuose. Taikant šiuolaikinius didelio jautrumo tyrimų metodus DNR metilinimo pokyčiai gali būti aptikti ne tik navikuose, bet ir ligonių organizmo skysčiuose ir pritaikyti ankstyvajai ligos diagnostikai.

Tyrimo tikslas. Ištirti DNR metilinimo pokyčius priešinės liaukos vėžiu sergančiųjų šlapimo nuosėdose, siekiant atrinkti informatyviausius neinvazinius ligos biožymenis.

Tyrimo medžiaga ir metodai. Realaus laiko metilinimui jautrios PGR metodu buvo tiriamas *RASSF1*, *RARB* ir *GSTP1* genų promotoriaus DNR metilinimas 34 sergančiųjų antros ar trečios stadijos priešinės liaukos vėžiu šlapimo mėginiuose.

Rezultatai. Bent vieno geno metilinimas nustatytas 28 ligonių šlapimo nuosėdose (tyrimo jautrumas – 82 proc.). Genas *RASSF1* buvo metilintas 71 proc. (24 iš 34), *RARB* – 44 proc. (15 iš 34), *GSTP1* – 3 proc. (1 iš 34) atvejų. Didelis genų *RARB* ir *RASSF1* metilinimo intensyvumas (⩾50 proc.) nustatytas atitinkamai 40 proc. ir 20 proc. atvejų. Nustatytas statistiškai patikimas (p=0,01) ryšys tarp geno *RARB* metilinimo ir naviko diferenciacijos lygio pagal *Gleason* skalę. Bent vieno geno metilinimas dažniau (p=0,02) nustatytas vyresnio amžiaus ligonių grupėje.

Išvados. Tyrimas rodo didelį DNR metilinimo biožymenų, ypač *RASSF1* ir *RARB* genų, metilinimo jautrumą ir tinkamumą ankstyvajai neinvazinei priešinės liaukos vėžio diagnostikai.

References

1. Garcia M, Jemal A, Ward EM, Center MM, Hao Y, Siegel RL, et al. Global Cancer Facts & Figures 2007. Atlanta, GA: American Cancer Society; 2007.
2. Smailytė G, Kurtinaitis J. Cancer mortality differences among urban and rural residents in Lithuania. BMC Public Health 2008;8:56.
3. Ahmed H. Promoter methylation in prostate cancer and its application for the early detection of prostate cancer using serum and urine samples. Biomark Cancer 2010;2010:17-33.
4. Phe V, Cussenot O, Roupret M. Methylated genes as potential biomarkers in prostate cancer. BJU Int 2010;105:1364-70.
5. Schulz WA, Hoffmann MJ. Epigenetic mechanisms in the biology of prostate cancer. Semin Cancer Biol 2009;19(3): 172-80.
6. Ellinger J, Muller SC, Stadler TC, Jung A, von Ruecker A, Bastian PJ. The role of cell-free circulating DNA in the diagnosis and prognosis of prostate cancer. Urol Oncol 2011;29(2):124-9.
7. Goessl C, Krause H, Muller M, Heicappell R, Schrader M, Sachsinger J, et al. Fluorescent methylation-specific polymerase chain reaction for DNA-based detection of prostate cancer in bodily fluids. Cancer Res 2000;60:5941-5.
8. Goessl C, Muller M, Heicappell R, Krause H, Miller K. DNA-based detection of prostate cancer in blood, urine, and ejaculates. Ann N Y Acad Sci 2001;945:51-8.
9. Hoque MO, Topaloglu O, Begum S, Henrique R, Rosenbaum E, Van Criekinge W, et al. Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. J Clin Oncol 2005;23:6569-75.
10. Jeronimo C, Usadel H, Henrique R, Silva C, Oliveira J, Lopes C, et al. Quantitative GSTP1 hypermethylation in bodily fluids of patients with prostate cancer. Urology 2002;60:1131-5.
11. Suh CI, Shanafelt T, May DJ, Shroyer KR, Bobak JB, Crawford ED, et al. Comparison of telomerase activity and GSTP1 promoter methylation in ejaculate as potential screening tests for prostate cancer. Mol Cell Probes 2000;14:211-7.
12. Cairns P, Esteller M, Herman JG, Schoenberg M, Jeronimo C, Sanchez-Cespedes M, et al. Molecular detection of prostate cancer in urine by GSTP1 hypermethylation. Clin Cancer Res 2001;7:2727-30.
13. Gonzalgo ML, Pavlovich CP, Lee SM, Nelson WG. Prostate cancer detection by GSTP1 methylation analysis of post-

biopsy urine specimens. Clin Cancer Res 2003;9:2673-7.
14. Woodson K, O'Reilly KJ, Hanson JC, Nelson D, Walk EL, Tangrea JA. The usefulness of the detection of GSTP1 methylation in urine as a biomarker in the diagnosis of prostate cancer. J Urol 2008;179:508-11.
15. Payne SR, Serth J, Schostak M, Kamradt J, Strauss A, Thelen P, et al. DNA methylation biomarkers of prostate cancer: confirmation of candidates and evidence urine is the most sensitive body fluid for non-invasive detection. Prostate 2009;69:1257-69.
16. Roupret M, Hupertan V, Yates DR, Catto JWF, Rehman I, Meuth M, et al. Molecular detection of localized prostate cancer using quantitative methylation-specific PCR on urinary cells obtained following prostate massage. Clin Cancer Res 2007;13:1720-5.
17. Vener T, Derecho C, Baden J, Wang H, Rajpurohit Y, Skelton J, et al. Development of a multiplexed urine assay for prostate cancer diagnosis. Clin Chem 2008;54:874-82.
18. Rogers CG, Gonzalgo ML, Yan G, Bastian PJ, Chan DY, Nelson WG, et al. High concordance of gene methylation in post-digital rectal examination and post-biopsy urine samples for prostate cancer detection. J Urol 2006;176:2280-4.
19. Jarmalaitė S, Jankevičius F, Kurgonaitė K, Sužiedelis K, Mutanen P, Husgafvel-Pursiainen K. Promoter hypermethylation in tumour suppressor genes shows association with stage, grade and invasiveness of bladder cancer. Oncology 2008;75:145-51.
20. Costa VL, Henrique L, Jeronimo C. Epigenetic markers for molecular detection of prostate cancer. Dis Markers 2007;23:31-41.
21. Gonzalgo ML, Yegnasubramanian S, Yan G, Rogers CG, Nicol TL, Nelson WG, et al. Molecular profiling and classification of sporadic renal cell carcinoma by quantitative methylation analysis. Clin Cancer Res 2004;10:7276-83.
22. Bastian PJ, Ellinger J, Wellmann A, Wernert N, Heukamp LC, Muller SC, et al. Diagnostic and prognostic information in prostate cancer with the help of a small set of hypermethylated gene loci. Clin Cancer Res 2005;11:4097-106.
23. Sunami E, Shinozaki M, Higano CS, Wollman R, Dorff TB, Tucker SJ, et al. Multimarker circulating DNA assay for assessing blood of prostate cancer patients. Clin Chem 2009;55:559-67.
24. Jeronimo C, Henrique R, Hoque MO, Ribeiro FR, Oliveira J, Fonseca D, et al. Quantitative RARbeta2 hypermethylation: a promising prostate cancer marker. Clin Cancer Res 2004;10:4010-4.
25. Kwabi-Addo B, Wang S, Chung W, Jelinek J, Patierno SR, Wang BD, et al. Identification of differentially methylated genes in normal prostate tissues from African American and Caucasian men. Clin Cancer Res 2010;16:3539-47.

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