Urine molecular biomarkers for detection and follow-up of 2 small renal masses

de Alina Petre

Data depunerii: 22-nov.-2022 07:16AM (UTC+0100)
ID-ul depunerii: 1961052881
Numele fişierului: ijms-2068048-peer-review-v1.pdf (997.66K)
Numărul cuvintelor: 6528
Numărul caracterelor: 33389

International Journal of *Molecular Sciences*

MDPI

Article

Urine molecular biomarkers for detection and follow-up of small renal masses

Algirdas Žalimas[1,2‡], Raimonda Kubiliūtė[1,2‡], Kristina Žukauskaitė[1,2], Rasa Sabaliauskaitė[1], Mantas Trakymas[1], Simona Letautienė[1,3], Edita Mišeikytė Kaubrienė[1,3], Jurgita Ušinskienė[1], Albertas Ulys[1] and Sonata Jarmalaitė[1,2*]

1 National Cancer Institute, Vilnius, Lithuania.
2 Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania.
3 Faculty of Medicine, Vilnius University, Vilnius, Lithuania
* Correspondence: author: Prof. Sonata Jarmalaite , National Cancer Institute, Santariskiu 1, LT-08406 Vilnius, Lithuania, sonata.jarmalaite@nvi.lt

Abstract: Active surveillance (AS) is the best strategy for small renal masses (SRMs) management, however reliable methods for early detection and disease aggressiveness prediction are urgently needed. The present study aimed to validate novel DNA methylation biomarkers for non-invasive SRMs detection and prognosis. The levels of methylated *TFAP2B*, *TAC1*, *PCDH8*, *ZNF677*, *FLRT2*, and *FBN2* were evaluated in 165 serial urine samples prospectively collected from 39 patients diagnosed with SRM, specifically renal cell carcinoma (RCC), before and during the AS via quantitative methylation-specific polymerase chain reaction. Voided urine samples from 92 asymptomatic volunteers were used as control. Significantly higher methylated *TFAP2B*, *TAC1*, *PCDH8*, *ZNF677*, and *FLRT2* levels and/or frequencies were detected in SRM patients' urine samples as compared to the control. The highest diagnostic power (AUC=0.74) was observed for the 4-biomarkers panel with 92% sensitivity and 52% specificity. Methylated *PCDH8* level positively correlated with SRM size at diagnosis, while *TFAP2B* had the opposite effect and was related to SRM progression. To sum up, SRMs contribute significantly to the amount of methylated DNA detectable in urine, that might be used for very early RCC detection. Moreover, *PCDH8* and *TFAP2B* methylation has the potential as prognostic biomarkers for SRMs.

Keywords: Small renal mass; Active surveillance; Renal cell carcinoma; Renal mass biopsy; Prognostic markers

Citation: Žalimas, A.; Kubiliūtė, R.; Žukauskaitė, K.; Sabaliauskaitė, R.; Trakymas, M.; Letautienė, S.; Kaubrienė, E.M.; Ušinskienė, J.; Ulys, A.; Jarmalaitė, S. Urine molecular biomarkers for detection and follow-up of small renal masses. **2022**, *23*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Received: date
Accepted: date
Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Small renal masses (SRMs) are defined as incidentally detected renal tumors less than or equal to 4 cm in diameter which is usually consistent with stage T1a renal cell carcinoma (RCC) [1]. To date in clinical practice, there are no specific tests for SRM detection, and although the majority of patients are diagnosed with localized kidney cancer, the true incidence of SRMs is unknown [2,3]. Despite earlier detection and treatment, epidemiologic studies have demonstrated stable RCC mortality, reflecting possible overdiagnosis and overtreatment [3,4]. Adequate clinical management of SRMs is important despite their slow growth and low metastatic potential. Surgical resection or nephrectomy remains the main treatment strategy. However, almost 20–30% of SRMs that are suspicious for malignancy by preoperative imaging reveal to be benign on final pathological examination after surgery [5]. Moreover, most incidental tumors are detected in elderly persons with comorbidities for whom, the surgical treatment risk outweighs the low oncologic risk. A possible alternative is percutaneous ablation of the SRM, but there is still a risk of complications or local recurrence [6].

Active surveillance (AS) strategy provides the ability to delay an active treatment and is the best strategy for SRMs management, which oncologic safety is reported by

numerous studies [7-12]. However, there is a possibility for metastatic progression (~ 2% of cases) [13] and rapid tumor growth, causing delayed interventions and/or additional treatment [7]. Without histological assessment, the only parameter that helps to predict the aggressiveness of SRMs is their growth rate, which is observed by periodic clinical and radiological evaluation [14]. Thus far, there are no available molecular biomarkers that could assist in timely SRM detection and accurately predict the progression of SRMs. Thus, novel molecular markers and genomic classifiers, containing molecular information from RCC (epi)genome, are urgently needed for the careful selection of patients with SRMs eligible for AS and disease aggressiveness prediction.

Changes in DNA methylation occur early during renal carcinogenesis [15], suggesting their suitability as biomarkers for early diagnosis of the disease. Aberrant DNA methylation is a stable modification modulating gene expression. DNA methylation profiles can be associated with the various clinical subgroups of kidney cancer and predict the aggressiveness of the tumor [16]. Moreover, these epigenetic marks are amenable for the detection in the body fluids such as urine by conventional and inexpensive PCR methods. Thus, it may serve as a non-invasive biomarker that could provide clinicians with rapid, objective, and accurate tools for the detection and follow-up of renal tumors. Despite efforts made [17,18], no studies are reporting on non-invasive methylated DNA biomarkers for SRMs [19].

In this study, a set of previously identified putative renal cancer-specific DNA methylation biomarkers [20] were investigated in the urine samples of patients diagnosed with SRMs and treated by AS, aiming at the evaluation of the suitability of these biomarkers for the detection of SRMs and tumor growth prediction during monitoring of the disease.

2. Methods

2.1. Patient selection and biosamples

SRMs patients inclusion criteria encompass (1) patients older than 18 yr; (2) incidental diagnosis at imaging (ultrasonography, CT, MRI) of a solid renal mass <4 cm in maximum diameter (pT1aN0M0); (3) histologically confirmed RCC by percutaneous needle biopsy at diagnosis; all RCC subtypes are eligible for the study; (4) patients inappropriate for active treatment due to advanced age, or co-morbidity, or choosing to avoid active treatment and signed informed consent. Patients' exclusion criteria: (1) an estimated patients life expectancy of <1 yr; (2) patients on concurrent systemic therapy for malignancy; (3) patients having a hereditary renal cancer syndrome, and/or had a nondiagnostic (noninformative)or benign biopsy.

The patients were included between March 2018 and 2022 and monitored at the National Cancer Institute (Vilnius, Lithuania). Approval from the Lithuanian Bioethics Committee was obtained, and all patients gave informed consent for participation. The prospective trial included 125 patients with SRMs, of whom 71 were biopsied. A percutaneous biopsy of the renal mass was performed to histologically confirm the diagnosis of RCC. The histopathologic evaluation of the cores was performed with hematoxylin and eosin (H&E) staining and specific histochemical and immunohistochemical stains when needed to identify specific subtypes of RCC. Among 46 patients with proven SRM RCC, 39 were eligible for the biomarker study (**Fig. 1**). Among these cases, 28 (71.8%) were biopsy proved clear cell RCC (ccRCC), 7 (17.9%) papillary RCC (pRCC), 3 (7.7%) chromophobe RCC (chRCC) and 1 (2.6%) had mixed ccRCC and pRCC histology. Ten (27.8%) of ccRCC and pRCC tumors had ISUP grade 1, twenty-three (63.9%) grade 2 and for the remaining three (8.3%) cases, the ISUP grade was undetermined. The median patient age at the diagnosis was 78 (IQR 72-81) yr. The median follow-up time was 22 (IQR 12-27) months. Of the patients, 77% were followed for at least 1 yr, 33% for at least 2 yr, and 13% for at least 3 yr. All patient's clinical and demographic data are provided in **Table S1**.

2.2. Imaging Examination and Measurement of Lesions

During the study/ follow-up, 165 images were obtained, of which 146 (88.5%) were scanned with computed tomography (CT), 16 (9.7%) were scanned with magnetic resonance imaging (MRI), and 3 (1.8%) were obtained by ultrasound (US). All images were evaluated by expert radiologists.

Serial imaging with CT, MRI, or US has been performed approximately at 3, 6, and 12 months till 3 years. Tumor size was measured by maximal axial diameter. Tumor volume was calculated using the formula for ellipsoid volume calculation: 0.5326×X×Y×Z. Tumor growth was reported in mm^3/year (volume growth rate), mm/year referring to the largest 2 diameters at 90 degrees to each other (linear growth rate). Tumor progression was defined by (1) large size (sustained maximum diameter >4 cm in at least two measurements within 15 mo) and/or (2) rapid growth (doubling of tumor volume over any one-year period). These criteria for local progression have been reported previously [21] and represent conservative expert opinion as triggers for treatment consideration [22].

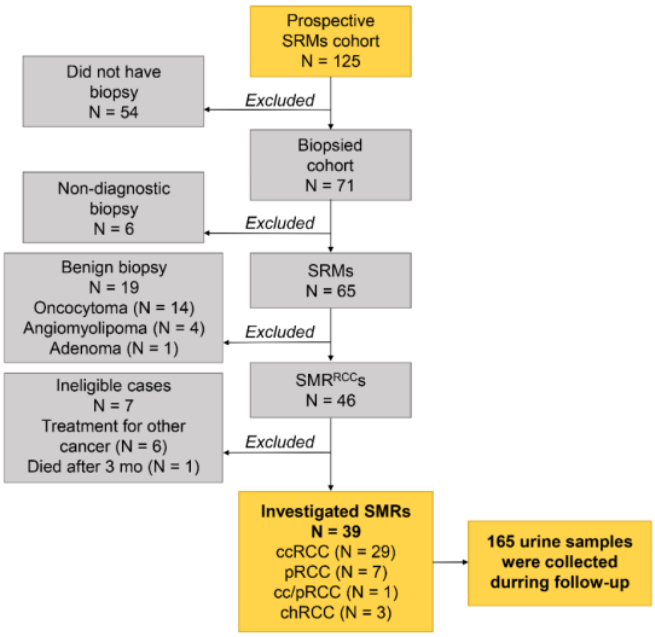


Figure 1. Consort diagram of the cohorts of patients with SRMs managed by active surveillance. SRM – small renal mass; RCC – renal cell carcinoma; ccRCC – clear cell RCC; pRCC – papillary RCC; chRCC – chromophobe RCC; mo – month.

2.3. *Urinary DNA purification*

Serial urine samples were collected from the 39 patients diagnosed with SRMs at different AS time points (165 samples in total): at the beginning of surveillance, and during follow-up. A voided urine sample from 92 asymptomatic volunteers from the previous study [20] was used as asymptomatic control (ASC).

Urine samples (up to 100 ml) were centrifuged at 2000 × *g* for 15 min at 4 °C. The supernatant was removed and urine sediments were washed twice with phosphate-buffered saline. DNA from urine sediments (~2 ml) was released by treating samples for 18 h at 55 °C with 10 µl of proteinase K (Thermo Scientific™, Thermo Fisher Scientific, Wilmington, DE, USA) in 500 µl of lysis buffer, consisting of 175 mM EDTA, 750 mM NaCl, 100 mM Tris-HCl (pH = 8.0), and 1% sodium dodecyl sulfate (all from Carl Roth,

Karlsruhe, Germany). DNA was purified following the standard phenol-chloroform protocol as previously reported [23].

2.4. Quantitative DNA methylation analysis

Four hundred ng of purified DNA were bisulfite-modified for DNA methylation analysis of the target genes, using EZ DNA Methylation™ Kit (Zymo Research, Irvine, CA, USA) following manufacturer's instructions, except the primary incubation of samples was performed at 42 °C for 15 min. Modified DNA samples were analyzed immediately or stored at ≤-20 °C.

The bisulfite-modified DNA was used as a template for the quantitative methylation-specific polymerase chain reaction (QMSP). As the endogenous control gene, *ACTB* was included in each assay to normalize DNA input [24]. The QMSP primers and hydrolysis probes, specific for the methylated DNA of the genes *ZNF677*, *FBN2*, *PCDH8*, *TFAP2B*, *TAC1*, and *FLRT2* were designed as described previously [20] and ordered from Metabion (Martinsried, Germany). QMSP was performed in triplicates for each set of primers in separate wells.

Methylation-positive (MC) and no-template controls (NTC) were routinely included in all experiments for each primer pair. *In vitro* fully methylated and bisulfite-modified human leukocyte DNA served as the methylated control (MC). The reaction mixture of a 20-µL final volume consisted of 1× TaqMan® Universal Master Mix II, no UNG (Applied Biosystems™), 300 nM of each primer, 50 nM of the probe, and 10 ng of bisulfite-converted DNA. All assays were performed using the ViiA7 qPCR System (Applied Biosystems™) under the following regime: 95°C for 10 min followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. A run considered valid where MCs provided a positive signal and the NTC gave no amplification product. The methylation intensity of a particular gene was estimated as a percentage of the MC.

2.5. Statistical analysis

Statistical analysis was performed using MedCalc® v14.0 software (MedCalc Software, Ostend, Belgium), STATISTICA™ v8.0 (StatSoft, Tulsa, OK, USA), and GraphPad Prism v8.0.1 (GraphPad Software, Inc.). For quantitative variables, a nonparametric Mann–Whitney U test was applied for the comparison between the two groups. Spearman's (Rs) rank correlation coefficients were calculated to test the associations between two continuous variables. The diagnostic value of biomarkers to distinguish SRMs and ASC was evaluated by performing receiver operating characteristic (ROC) curve analysis and estimating the area under the curve (AUC). The diagnostic test's performance parameters – sensitivity and specificity were obtained based on the Youden index for the selection of optimal cut-off value. For various combinations of biomarkers, logistic regression analysis was applied. Differences and associations were considered statistically significant at $P < 0.050$.

3. Results

3.1. Tumor growth dynamics

Out of 39 SRMs included in the biomarker study, 11 (28.2%) lesions had progressed. Out of them, 9 (81.2%) SRMs had progressed according to the large tumor size on follow-up (> 4 cm in maximal diameter) and 2 (18.2%) according to the rapid tumor growth (doubling in volume). All progressed cases had clear cell histology, of which 3 (27.3%) ISUP grade 1 and 8 (72.7%) ISUP grade 2. Two cases progressed within the first year of AS, 5 cases – during the second year of follow-up, and the remaining 4 – during the third year or later. Four patients were treated by partial or radical nephrectomy, 4 – by ablation, while for the remaining 3 the AS was continued due to the contraindications for active treatment (age and comorbidities). During follow-up, no cases developed metastatic disease, however, one tumor progressed to T3a clinical stage.

The median maximal tumor diameter at the diagnosis was 2.3 (range 1.0-3.7) cm, while at the last scan – 2.8 (range 1.2-5.2) cm. Similarly, the median tumor volume at the diagnosis was 4.8 (range 0.5-20.9) cm^3, while at the last scan – 10.1 (range 0.5-44.1) cm^3. During AS period, the average growth rate in maximal tumor diameter of all tumors was 18.1% (0.4 cm), while the volume growth rate was 62.9% (4.5 cm^3) (**Fig. 2 A**).

Of importance, during AS the average growth rate in maximal tumor diameter of non-progressing tumors was 1.9% (<0.1 cm)), while the same measure of progressing tumors was almost 30 fold large and reached 52.8% (1.3 cm). Similarly, the volume growth rate of non-progressing tumors was 8.9% (0.6 cm^3), while of progressing tumors 168.5% (13.5 cm^3). These differences were highly statistically significant ($P < 0.001$).

While comparing clinical demographic variables, no significant differences between progressing and non-progressing tumors were observed at the initial scan except for tumor histology (**Table S2**). A significantly larger size of progressing tumors compared to the non-progressing ones was observed from the third scan, i.e., after ~12 months of AS ($P < 0.005$; **Fig. 2 C-D**).

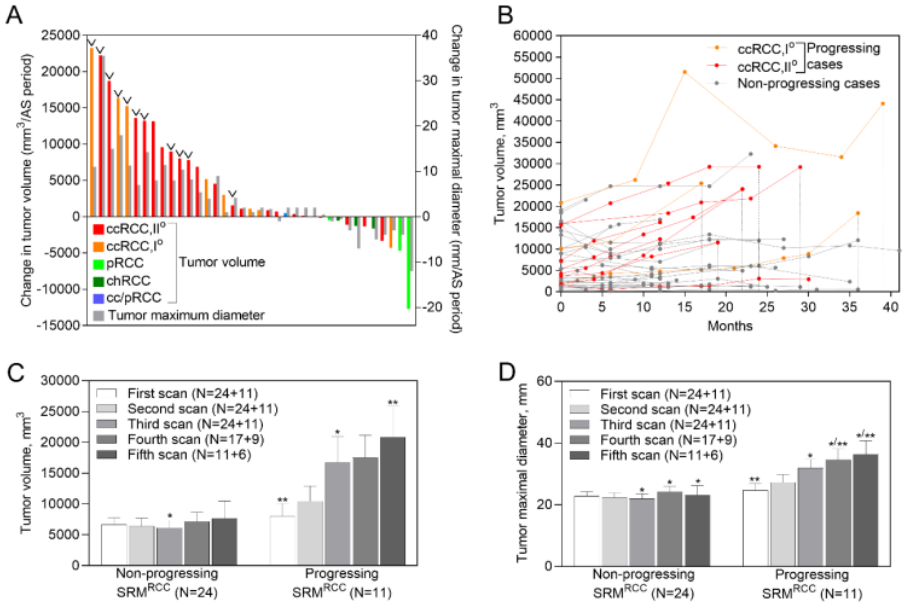


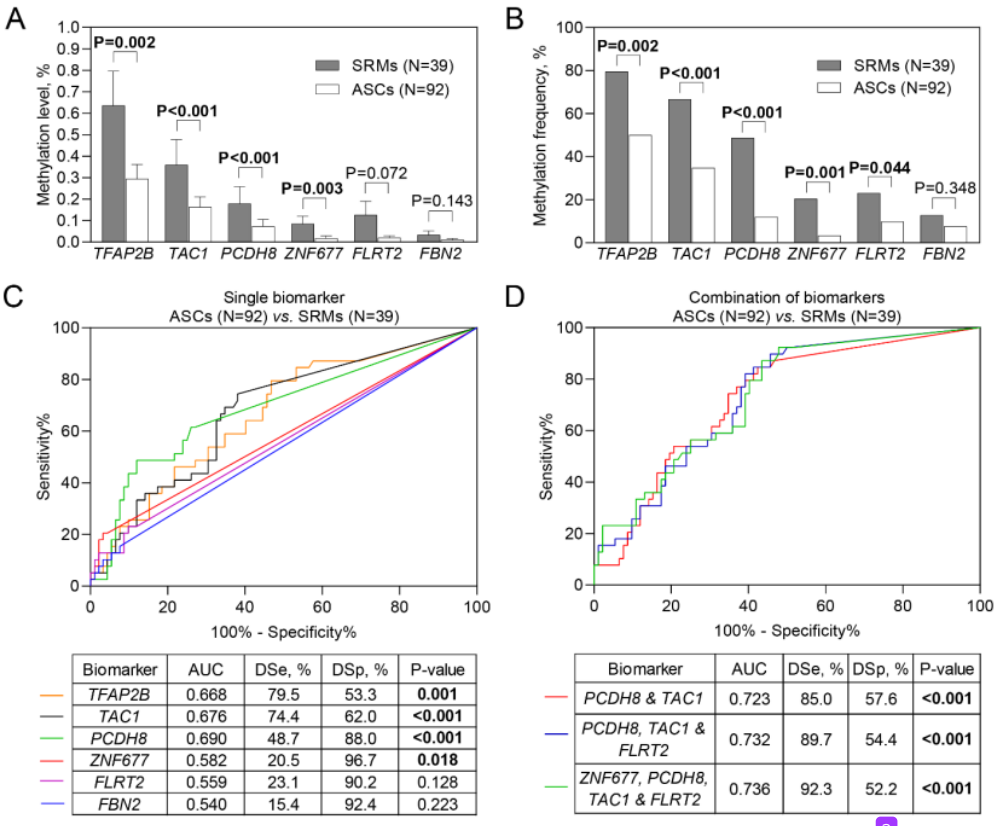
Figure 2. Growth kinetics of biopsy confirmed SRM RCC. A – Waterfall plots of tumor growth rate during all AS periods. B – Tumors growth kinetic at different time-points of AS period. C–D – average tumor size at different scans. Whiskers indicate the standard error of the mean. ∨ indicate progressing cases; * indicate significant differences between the groups (Non-progressing *vs.* Progressing); ** indicate significant differences within the group. SRM – small renal masses; (cc)RCC – (clear cell) Renal cell carcinoma; mo – months; AS – active surveillance.

3.2. Diagnostic potential of the urinary biomarkers

DNA methylation of the six genes *ZNF677, FBN2, PCDH8, TFAP2B, TAC1,* and *FLRT2* was analyzed quantitatively in urine sediment samples, collected from the patients diagnosed with SRMs (N = 39) and asymptomatic (N = 92) control. Methylated DNA levels and/or frequencies of *TFAP2B, TAC1, PCDH8, ZNF677,* and *FLRT2* were higher among the patients diagnosed with SRMs as compared to ASC (all $P < 0.050$; **Fig.3 A, B**).

Assessing the diagnostic potential of each of the genes separately, *PCDH8* (AUC = 0.69; $P < 0.001$) had the highest diagnostic values, with sensitivity and specificity equal to

48.7% and 88.0%, respectively (**Fig. 3C**). The analysis of potential biomarkers in all possible combinations was performed, and the four-biomarkers panel, particularly, *ZNF677, PCDH8, TAC1 & FLRT2* was characterized with the best diagnostic potential (AUC = 0.74) reaching 92% of sensitivity and 52% of specificity (**Fig. 3D; Table S3.**).



Biomarker	AUC	DSe, %	DSp, %	P-value
TFAP2B	0.668	79.5	53.3	**0.001**
TAC1	0.676	74.4	62.0	**<0.001**
PCDH8	0.690	48.7	88.0	**<0.001**
ZNF677	0.582	20.5	96.7	**0.018**
FLRT2	0.559	23.1	90.2	0.128
FBN2	0.540	15.4	92.4	0.223

Biomarker	AUC	DSe, %	DSp, %	P-value
PCDH8 & TAC1	0.723	85.0	57.6	**<0.001**
PCDH8, TAC1 & FLRT2	0.732	89.7	54.4	**<0.001**
ZNF677, PCDH8, TAC1 & FLRT2	0.736	92.3	52.2	**<0.001**

Figure 3. Diagnostic characteristics of the investigated biomarkers. A – methylation levels of *ZNF677, FBN2, PCDH8, TFAP2B, TAC1,* and *FLRT2* in the urine samples from patients diagnosed with small renal masses (SRMs; pT1a; ≤4 cm) and asymptomatic controls (ASC). B - frequencies of methylated *ZNF677, FBN2, PCDH8, TFAP2B, TAC1,* and *FLRT2* among the patients diagnosed with SRMs and ASCs. C - ROC curve analysis for single biomarkers and D - the combination of biomarkers in discriminating patients with SRMs and ASC. The bars represent the average DNA methylation levels with a standard error of the mean. ROC – receiver operating characteristic; AUC – area under the curve; DSe – diagnostic sensitivity; DSp – diagnostic specificity. Significant P values are in bold.

3.3. *Prognostic value of urinary biomarkers*

Aberrant methylation of the investigated genes was further analyzed according to the clinical and demographic variables of SRM cases. A significantly higher methylation level of *PCDH8* and *TFAP2B* was observed in females, compared to males (P = 0.009 and P = 0.042 respectively; **Table S4**). In addition, methylated levels of *TFAP2B* and *TAC1* had a tendency to correlate with glomerular filtration rate, while *ZNF677* and *PCDH8* were significantly related to the blood creatinine level (R_s = -0.33; P = 0.048 and R_s = -0.36; P = 0.028 respectively; **Table S4**), indicating the possible contribution of these genes to the kidney functions.

Most importantly, a significant association of methylated *PCDH8* level to tumor maximal diameter at diagnosis ($R_s = 0.35$; $P = 0.028$) as well as borderline significance to tumor volume ($R_s = 0.32$; $P = 0.051$) at diagnosis was identified (**Fig 4 A, B**). Meanwhile, *TFAP2B* methylation level was negatively associated with the changes in tumor maximal diameter and volume growth rate (**Fig. 4 C, D**). Moreover, a comparison of the progressing tumors with non-progressing SRMs revealed a lower level of methylated *TFAP2B* at SRM diagnosis ($P = 0.016$; **Fig. S1**), though no significant changes between different time-points during follow-up were detected (**Fig. S2**).

Assessment of correlations between the methylation levels of described genes and individual tumor growth dynamics revealed some positive associations (**Fig. 4G-H**), however, in most of the cases, the associations were quite stochastic and only partially reflected tumor growth (**Fig. S3**).

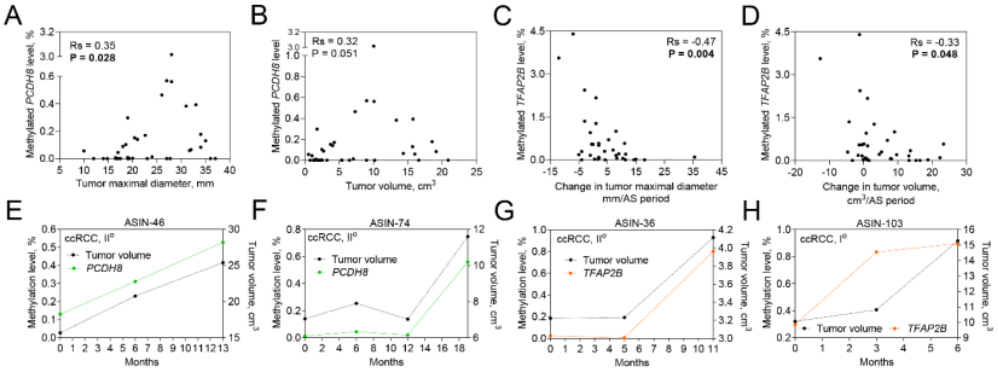


Figure 4. The association of investigated biomarkers with SRMs size and growth rate. A-B - Correlation between methylated *PCDH8* level and SRMs size at diagnosis; C-D – Correlation between methylated *TFAP2B* level and change in tumor size during AS period. E-H – associations between individual tumor growth dynamics and methylated *PCDH8* and *TFAP2B* levels. Rs – Spearman correlation coefficient. AS – active surveillance. ccRCC – clear cell renal cell carcinoma. ASIN depicts the patient's ID. Significant P-values are in bold.

4. Discussion

The present study of urinary biomarkers shows that SRMs contribute significantly to the amount of DNA detectable in urine and, therefore, DNA methylation might be used as an indicator of the formation of small malignant masses in the kidney. In urine samples from the cases with SRMs significantly higher methylation levels and/or frequencies of *TFAP2B*, *TAC1*, *PCDH8*, *ZNF677*, and *FLRT2* were detected in comparison to controls in support to our previous observations of the diagnostic potential of these DNA methylation biomarkers in RCC [20]. Besides, the methylation changes in *PCDH8* and *TFAP2B* were related to the amount of tumor mass or the dynamics of tumor growth, thus may potentially supplement imaging data on SRMs' growth.

AS is a sufficiently safe and reasonable management strategy for the elderly and comorbid patients with SRM suspicious of a clinical T1a RCC. Unfortunately, with or without surgical treatment, there is still a potential for metastatic progression [13]. Optimal management of SRMs should balance the need for treatment, as around 25% of all SMRs are benign lesions, with the goal of preserving renal function as far as possible and avoiding the risk of overtreatment [25]. In the present study, core biopsies were conclusive in 91.5% (65 out of 71) cases, identifying 29.2% (19 out of 65) benign masses and 70.8% (46 out of 65) RCCs. Thus, the sensitivity of needle biopsy was slightly lower than previously reported [26-28]. Currently, the best way to overcome false negative results is by repeated biopsy [26,28], however, next to the high price, it also provides many difficulties for patients and radiologists, especially in the case of SRMs. Although a previous study

established DNA methylation-based identifiers for needle biopsy samples [17], a large amount of variables included in this identifier is of limited clinical use due to the high cost and intricate interpretation. Currently, simple liquid biopsy-based methods are coming to the clinics as an additional tool for radiological follow-up of SMRs.

In our study, in urine samples from the patients with SRMs, out of five SRM-specific biomarkers, the highest diagnostic power (AUC=0.69) with 49% of sensitivity and 88% of specificity was established for *PCDH8*. These data are in support of our previous findings on the high diagnostic value of this biomarker in RCC [20]. Interestingly, the sensitivities of methylated *TFAP2B* and *TAC1* for RCC SRMs were even higher than that of needle biopsy, which possibly is the sequel of RCC heterogeneity which is highly overlooked in the single biopsy studies [29-31]. Moreover, our 4-biomarkers panel was able to detect > 90% of small renal tumors, although the specificity only slightly exceeded 50%. To the best of our knowledge, the present study for the first time analyzed DNA methylation in the urine samples of SRM patients and revealed the possibility to use it as a non-invasive tool for the detection of extremely small (<4 cm in the maximal diameter) malignant renal mass.

There is an ongoing debate about the treatment of SRMs due to the low rate of progression observed during AS [32]. Till now, serial imagining and tracking the growth rate of SRMs is the only strategy for the surveillance of patients non-eligible for surgical treatment. In this study, among investigated 39 cases, 28% have progressed demonstrating a considerably higher growth rate (13.5 cm3/AS) as compared to non-progressing ones (0.6 cm3/AS). However, a significant difference in tumor size among progressing and non-progressing tumors was observed only after about 12 months of AS, but such a tendency was not evident at the diagnosis. While the molecular biomarker analysis in the urine samples of SRM RCC patients at diagnosis demonstrated the positive correlation between methylated *PCDH8* level and tumor size at diagnosis, confirming our previous report and its prognostic potential [20]. Some other studies reported *PCDH8* as a tumor suppressor, inhibiting cell proliferation, and migration, and inducing apoptosis, and thus are inactivated by promoter methylation in some carcinomas [33-35]. Besides, a significantly higher methylated *TFAP2B* level among non-progressing cases compared to progressing ones at SRM diagnosis was observed as well. Lower methylated *TFAP2B* levels among progressive tumors may be partially explained by its contribution to the activation of the WNT/β-catenin signaling pathway, promoting cell proliferation and RCC progression [36-38]. The results suggest the potential of DNA methylation biomarkers to predict SRM progression at disease diagnosis, thus stratifying patients into eligible and ineligible for AS, however further investigations in pursuance to applicate such prognostic biomarkers in clinical practice are needed.

Along with the significant impact of the study in the field of AS of SRMs, the investigation has some important shortcomings as well. Firstly, a small number of patients recruited in the study prevented us from performing a more comprehensive statistical analysis. Secondly, next to the asymptomatic control, urine samples from the benign SRMs cases would be highly desirable to prove the suitability of the investigated biomarkers for non-invasive specific SRM detection. Thirdly, stochasticity in tumor growth is partially possibly influenced by different imagining methods used (due to the toxicity of CT in some cases) probably biases the tumor growth measurements that may affect statistical analysis as well. Nonetheless, the novelty of using DNA methylation biomarkers with presumable diagnostic and prognostic value for SRM/RCC and amenable for non-invasive urine-based detection doubtless will stimulate further investigations in this field.

5. Conclusions

To sum up, the RCC-specific aberrant methylation of *TFAP2B*, *TAC1*, *PCDH8*, *ZNF677*, and *FLRT2* is detectable in urine from cases with SRM and is potentially suitable for non-invasive early detection and follow-up of the disease. Moreover, *PCDH8* and

TFAP2B methylation show some potential as prognostic biomarkers for SRM patients' active surveillance, although further thorough investigations are needed.

Author Contributions: A.Ž. performed the patient selection, collect clinical data, and performed their analysis, drafted the manuscript; R.K., K.Ž. performed the DNA methylation analysis, analyzed the experimental and clinical data, and drafted the manuscript. R.S. coordinated sample and data collection; M.T., S.L., E.M.K., J.U. performed SRM biopsies and/or radiological examinations of the patients; coordinated sample and data collection; A.U. implemented patient selection and follow-up; S.J. designed the research, supervised the analysis of the clinical and experimental data, and revised the manuscript critically for important intellectual content. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work is appropriately investigated and resolved.

Funding: This work was funded by the 2014–2020 European Union Structural Funds according to the activity "Intelligence. Joint science-business projects" grant No. J05-LVPA-K-04-0029.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Lithuanian Bioethics Committee (Nr.: 158200-17-952-457).

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data supporting the results reported in the article is available from the corresponding author upon a reasonable request.

Conflicts of Interest: R.K. and S.J. are inventors of the patent application for DNA methylation biomarkers (application No. PCT/IB2017052532; filed by Vilnius University). JSC "ThermoPharmaBaltic" has licensed the patent. The remaining authors declare that they have no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. [illegible] IS, Aron M, Gervais DA, Jewett MA. Clinical practice. Small renal mass. N Engl J Med. 2010 Feb 18;362(7):624-34.
2. Sanchez A, Feldman AS, Hakimi AA. Current Management of Small Renal Masses, Including Patient Selection, Renal Tumor Biopsy, Active Surveillance, and Thermal Ablation. J Clin Oncol. 2018;36(36):3591-3600.
3. Sohlberg EM, Metzner TJ, Leppert JT. The Harms of Overdiagnosis and Overtreatment in Patients with Small Renal Masses: A Mini-review. Eur Urol Focus. 2019 Nov;5(6):943-945.
4. Znaor A, Lortet-Tieulent J, Laversanne M, Jemal A, Bray F. International variations and trends in renal cell carcinoma incidence and mortality Eur Urol. 2015;67(3):519-30.
5. Li C, Cuilleron M, Gentil-Perret A, Tostain J. Characteristics of image-detected solid renal masses: implication for optimal treatment. Int J Urol. 2004;11(2):63-7.
6. Gunn AJ, Gervais DA. Percutaneous ablation of the small renal mass-techniques and outcomes. Semin Intervent Radiol. 2014;31(1):33[illegible].
7. Menon AR, Hussein AA, Attwood KM, White T, James G, Xu B, Petroziello M, Roche CL, Kauffman EC. Active Surveillance for Risk Stratification of All Small Renal Masses Lacking Predefined Clinical Criteria for Intervention. J Urol. 2021;206(2):229-239. doi: 10.1097/JU.0000000000001714.
8. Whelan EA, Mason RJ, Himmelman JG et al.: Extended Duration of Active Surveillance of Small Renal Masses: A Prospective Cohort Study. J Urol, 2019;202: 57.
9. Gupta M, Alam R, Patel HD et al.: Use of delayed intervention for small renal masses initially managed with active surveillance. Urol Oncol, 2019;37: 18.
10. McIntosh AG, Ristau BT, Ruth K et al.: Active Surveillance for Localized Renal Masses: Tumor Growth, Delayed Intervention Rates, and >5-yr Clinical Outcomes. Eur Urol, 2018;74:157.
11. Paterson C, Yew-Fung C, Sweeney C et al.: Predictors of growth kinetics and outcomes in small renal masses (SRM </=4 cm in size): Tayside Active Surveillance Cohort (TASC) Study. Eur J Surg Oncol, 2017;43:1589.
12. Schiavina R, Borghesi M, Dababneh H et al.: Small renal masses managed with active surveillance: predictors of tumor growth rate after long-term follow-up. Clin Genitourin Cancer, 2015;13: e87.
13. Smaldone MC, Kutikov A, Egleston BL, Canter DJ, Viterbo R, Chen DY, Jewett MA, Greenberg RE, Uzzo RG. Small renal masses progressing to metastases under active surveillance: a systematic review and pooled analysis. Cancer. 2012 Feb 15;118(4):997-1006.
14. Sebastià C, Corominas D, Musquera M, Paño B, Ajami T, Nicolau C.Active surveillance of small renal masses. Insights Imaging. 2020;11(1):63.

15. Arai E, Kanai Y. Genetic and epigenetic alterations during renal carcinogenesis. Int J Clin Exp Pathol. 2010;4(1):58-73.
16. Chen W, Zhuang J, Wang PP, Jiang J, Lin C, Zeng P, Liang Y, Zhang X, Dai Y, Diao H. DNA methylation-based classification and identification of renal cell carcinoma prognosis-subgroups. Cancer Cell Int. 2019;19:185.
17. Chopra S, Liu J, Alemozaffar M, Nichols PW, Aron M, Weisenberger DJ, Collings CK, Syan S, Hu B, Desai M, Aron M, Duddalwar V, Gill I, Liang G, Siegmund KD. Improving needle biopsy accuracy in small renal mass using tumor-specific DNA methylation markers. Oncotarget. 2017 Jan 17;8(3):5439-5448. doi: 10.18632/oncotarget.12276.
18. Ibragimova I, Slifker MJ, Maradeo ME, Ba[illegible]nathy G, Dulaimi E, Uzzo RG, Cairns P. Genome-wide promoter methylome of small renal masses. PLoS One. 2013 Oct 24;8(10):e77309. doi: 10.1371/journal.pone.0077309.
19. Kubiliute R, Jarmalaite S. Epigenetic Biomarkers of Renal Cell Carcinoma for Liquid Biopsy Tests. Int J Mol Sci. 2021 Aug 22(16):8846. doi: 10.3390/ijms22168846.
20. Kubiliūtė R, Žukauskaitė K, Žalimas A, Ulys A, Sabaliauskaitė R, Bakavičius A, Želvys A, Jankevičius F, Jarmalaitė S. Clinical significance of novel DNA methylation biomarkers for renal clear cell carcinoma. J Cancer Res Clin Oncol. 2022 Feb;148(2):361-[illegible]. doi: 10.1007/s00432-021-03837-7.
21. Kunkle DA, Chen DY, Greenberg RE, Viterbo R, Uzzo RG. Metastatic progression of enhancing renal masses under active surveillance is associated with rapid interval growth of the primary tumor. J Urol 2008. https://doi.org/10.1016/S0022-[illegible]7(08)61097-6
22. Volpe A, Panzarella T, Rendon RA, Haider MA, Kondylis FI, Jewett MA. The natural history of incidentally detected small renal masses. Cancer 2004;100:738-45
23. Daniunaite K, Jarmalaite S, Kalinauskaite N, Petroska D, Laurinavicius A, Lazutka JR, Jankevicius F. Prognostic value of RASSF1 promoter methylation in prostate cancer. J Urol. 2014 Dec;192(6):1849-55.
24. Lehmann U, Langer F, Feist H, Glöckner S, Hasemeier B, Kreipe H. Quantitative assessment of promoter hypermethylation during breast cancer development. Am J Pathol. 2002;160(2):605-12.
25. Finelli A, Cheung DC, Al-Matar A, Evans AJ, Morash CG, Pautler SE, Siemens DR, Tanguay S, Rendon RA, Gleave ME, Drachenberg DE, Chin JL, Fleshner NE, Haider MA, Kachura JR, Sykes J, Jewett MAS. Small Renal Mass Surveillance: Histology-specific Growth Rates in a Biopsy-characterized Cohort. Eur Urol. 2020;78(3):460-467.
26. Leveridge MJ, Finelli A, Kachura JR, Evans A, Chung H, Shiff DA, Fernandes K, Jewett MA. Outcomes of small renal mass needle core biopsy, nondiagnostic percutaneous biopsy, and the role of repeat biopsy. Eur Urol 2011, 60:578-584.
27. Park SY, Park BK, Kim CK, Kwon GY. Ultrasound-guided core biopsy of small renal masses: diagnostic rate and limitations. J Vasc Interv Radiol. 2013 Jan;24(1):90-6.
28. Richard PO, Jewett MA, Tanguay S, Saarela O, Liu ZA, Pouliot F, Kapoor A, Rendon R, Finelli A. Safety, reliability and accuracy of small renal tumour biopsies: results from a multi-institution registry. BJU Int. 2017;119(4):543-549.
29. Mitchell TJ, Turajlic S, Rowan A, Nicol D, Farmery JHR, O'Brien T, Martincorena I, Tarpey P, Angelopoulos N, Yates LR, Butler AP, Raine K, Stewart GD, Challacombe B, Fernando A, Lopez JI, Hazell S, Chandra A, Chowdhury S, Rudman S, Soultati A, Stamp G, Fotiadis N, Pickering L, Au L, Spain L, Lynch J, Stares M, Teague J, Maura F, Wedge DC, Horswell S, Chambers T, Litchfield K, Xu H, Stewart A, Elaidi R, Oudard S, McGranahan N, Csabai I, Gore M, Futreal PA, Larkin J, Lynch AG, Szallasi Z, Swanton C, Campbell PJ; TRACERx Renal Consortium. Timing the Landmark Events in the Evolution of Clear Cell Renal Cell Cancer: TRACERx Renal. Cell. 2018;173(3):611-623.e17.
30. Turajlic S, Xu H, Litchfield K, Rowan A, Chambers T, Lopez JI, Nicol D, O'Brien T, Larkin J, Horswell S, Stares M, Au L, Jamal-Hanjani M, Challacombe B, Chandra A, Hazell S, Eichler-Jonsson C, Soultati A, Chowdhury S, Rudman S, Lynch J, Fernando A, Stamp G, Nye E, Jabbar F, Spain L, Lall S, Guarch R, Falzon M, Proctor I, Pickering L, Gore M, Watkins TBK, Ward S, Stewart A, DiNatale R, Becerra MF, Reznik E, Hsieh JJ, Richmond TA, Mayhew GF, Hill SM, McNally CD, Jones C, Rosenbaum H, Stanislaw S, Burgess DL, Alexander NR, Swanton C; PEACE; TRACERx Renal Consortium. Tracking Cancer Evolution Reveals Constrained Routes to Metastases: TRACERx Renal. Cell. 2018;173(3):581-594.e12.
31. Turajlic S, Xu H, Litchfield K, Rowan A, Horswell S, Chambers T, O'Brien T, Lopez JI, Watkins TBK, Nicol D, Stares M, Challacombe B, Hazell S, Chandra A, Mitchell TJ, Au L, Eichler-Jonsson C, Jabbar F, Soultati A, Chowdhury S, Rudman S, Lynch J, Fernando A, Stamp G, Nye E, Stewart A, Xing W, Smith JC, Escudero M, Huffman A, Matthews N, Elgar G, Phillimore B, Costa M, Begum S, Ward S, Salm M, Boeing S, Fisher R, Spain L, Navas C, Grönroos E, Hobor S, Sharma S, Aurangzeb I, Lall S, Polson A, Varia M, Horsfield C, Fotiadis N, Pickering L, Schwarz RF, Silva B, Herrero J, Luscombe NM, Jamal-Hanjani M, Rosenthal R, Birkbak NJ, Wilson GA, Pipek O, Ribli D, Krzystanek M, Csabai I, Szallasi Z, Gore M, McGranahan N, Van Loo P, Campbell P, Larkin J, Swanton C; TRACERx Renal Consortium. Deterministic Evolutionary Trajectories Influence Primary Tumor Growth: TRACERx Renal. Cell. 2018;173(3):595-610.e11.
32. Jewett MA, Mattar K, Basiuk J et al.: Active surveillance of small renal masses: progression patterns of early stage kidney cancer. Eur Urol, 2011;60: 39.
33. He D, Zeng Q, Ren G, Xiang T, Qian Y, Hu Q, Zhu J, Hong S, Hu G. Protocadherin8 is a functional tumor suppressor frequently inactivated by promoter methylation in nasopharyngeal carcinoma. Eur J Cancer Prev. 2012;21(6):569-75.
34. Yu JS, Koujak S, Nagase S, Li CM, Su T, Wang X, Keniry M, Memeo L, Rojtman A, Mansukhani M, Hibshoosh H, Tycko B, Parsons R. PCDH8, the human homolog of PAPC, is a candidate tumor suppressor of breast cancer. Oncogene. 2008;27(34):4657-65.
35. Zhang D, Zhao W, Liao X, Bi T, Li H, Che X. Frequent silencing of protocadherin 8 by promoter methylation, a candidate tumor suppressor for human gastric cancer. Oncol Rep. 2012;28(5):1785-91.

36. Wang L, Wang C, Wu T, Sun F. Long non-coding RNA TP73-AS1 promotes TFAP2B-mediated proliferation, metastasis and invasion in retinoblastoma via decoying of miRNA-874 3p. J Cell Commun Signal. 2020;14(2):193-205.
37. Valenta T, Hausmann G, Basler K. The many faces and functions of β- catenin EMBO J. 2012; 31(12):, 2714–2736.
38. Mo S, Su Z, Heng B, Chen W, Shi L, Du X, Lai C. SFRP1 Promoter Methylation and Renal Carcinoma Risk: A Systematic Review and Meta- Analysis. J Nippon Med Sch. 2018;85(2):78-86.

Urine molecular biomarkers for detection and follow-up of 2 small renal masses

RAPORT PRIVIND ORIGINALITATEA

50%	45%	45%	23%
INDICE DE SIMILITUDINE	SURSE DE PE INTERNET	PUBLICAŢII	LUCRĂRILE STUDENŢILOR

SURSE PRINCIPALE

1	epublications.vu.lt Sursă de pe Internet	14%
2	Kristina Daniunaite, Arnas Bakavicius, Kristina Zukauskaite, Ieva Raluseviciute et al. "Promoter Methylation of PRKCB, ADAMTS12, and NAALAD2 Is Specific to Prostate Cancer and Predicts Biochemical Disease Recurrence", International Journal of Molecular Sciences, 2021 Publicaţie	3%
3	link.springer.com Sursă de pe Internet	2%
4	Antonio Finelli, Douglas C. Cheung, Ashraf Al-Matar, Andrew J. Evans et al. "Small Renal Mass Surveillance: Histology-specific Growth Rates in a Biopsy-characterized Cohort", European Urology, 2020 Publicaţie	2%
5	Submitted to Konkuk University Lucrarea studentului	

		2%
6	www.wjgnet.com Sursă de pe Internet	2%
7	Raimonda Kubiliūtė, Kristina Žukauskaitė, Algirdas Žalimas, Albertas Ulys et al. "Clinical significance of novel DNA methylation biomarkers for renal clear cell carcinoma", Journal of Cancer Research and Clinical Oncology, 2021 Publicaţie	2%
8	insightsimaging.springeropen.com Sursă de pe Internet	1%
9	Zhuo T. Su, Hiten D. Patel, Mitchell M. Huang, Ridwan Alam et al. "Active Surveillance versus Immediate Intervention for Small Renal Masses: A Cost-Effectiveness and Clinical Decision Analysis", Journal of Urology, 2022 Publicaţie	1%
10	www.dovepress.com Sursă de pe Internet	1%
11	pure.uva.nl Sursă de pe Internet	1%
12	cslide.ctimeetingtech.com Sursă de pe Internet	1%
	coek.info	

13	Sursă de pe Internet	1 %
14	keck.usc.edu Sursă de pe Internet	1 %
15	res.mdpi.com Sursă de pe Internet	1 %
16	mdpi-res.com Sursă de pe Internet	1 %
17	edepot.wur.nl Sursă de pe Internet	1 %
18	clinicaltrials.gov Sursă de pe Internet	1 %
19	www.roswellpark.org Sursă de pe Internet	1 %
20	tau.amegroups.com Sursă de pe Internet	1 %
21	www.duo.uio.no Sursă de pe Internet	1 %
22	cellandbioscience.biomedcentral.com Sursă de pe Internet	1 %
23	Roberto Luigi Cazzato, Julien Garnon, Pierre De Marini, Pierre Auloge et al. "Is percutaneous image-guided renal tumour	<1 %

	ablation ready for prime time?", The British Journal of Radiology, 2020 Publicaţie	
24	www.nature.com Sursă de pe Internet	<1 %
25	www.vu.lt Sursă de pe Internet	<1 %
26	Sandra Seo Young Kim, Rodney Breau, Ranjeeta Mallick, Anil Kapoor et al. "Ultrasound Correlates Highly with Cross Sectional Imaging for Small Renal Masses in a Contemporary Cohort", Urology, 2022 Publicaţie	<1 %
27	discovery.ucl.ac.uk Sursă de pe Internet	<1 %
28	m.scirp.org Sursă de pe Internet	<1 %
29	www.scielo.br Sursă de pe Internet	<1 %
30	clinicalepigeneticsjournal.biomedcentral.com Sursă de pe Internet	<1 %
31	es.scribd.com Sursă de pe Internet	<1 %
32	repositorio-aberto.up.pt Sursă de pe Internet	<1 %

33	www.mdpi.com Sursă de pe Internet	<1 %
34	sciforum.net Sursă de pe Internet	<1 %
35	cris.maastrichtuniversity.nl Sursă de pe Internet	<1 %
36	www.spandidos-publications.com Sursă de pe Internet	<1 %
37	uroturk.org.tr Sursă de pe Internet	<1 %
38	pubmed.ncbi.nlm.nih.gov Sursă de pe Internet	<1 %
39	Submitted to AIIMS BHUBANESWAR Lucrarea studentului	<1 %
40	www.jbc.org Sursă de pe Internet	<1 %
41	researchrepository.ucd.ie Sursă de pe Internet	<1 %
42	Kristina Daniunaite, Sonata Jarmalaite, Neringa Kalinauskaite, Donatas Petroska et al. "Prognostic Value of RASSF1 Promoter Methylation in Prostate Cancer", The Journal of Urology, 2014 Publicaţie	<1 %

43	ascopubs.org Sursă de pe Internet	<1 %
44	www.biomedcentral.com Sursă de pe Internet	<1 %
45	Submitted to University of Reading Lucrarea studentului	<1 %
46	Jennifer Gordetsky, Marie-Lisa Eich, Manjula Garapati, Maria del Carmen Rodriguez Pena, Soroush Rais-Bahrami. "Active Surveillance of Small Renal Masses", Urology, 2018 Publicaţie	<1 %
47	Volpe, A.. "Techniques, Safety and Accuracy of Sampling of Renal Tumors by Fine Needle Aspiration and Core Biopsy", The Journal of Urology, 200708 Publicaţie	<1 %
48	backend.orbit.dtu.dk Sursă de pe Internet	<1 %
49	Kristina Daniunaite, Monika Dubikaityte, Povilas Gibas, Arnas Bakavicius et al. "Clinical significance of miRNA host gene promoter methylation in prostate cancer", Human Molecular Genetics, 2017 Publicaţie	<1 %
50	stemcellres.biomedcentral.com Sursă de pe Internet	<1 %

51	www.int-res.com Sursă de pe Internet	<1 %
52	www.oncotarget.com Sursă de pe Internet	<1 %
53	Nathalie Rioux-Leclercq, Pierre I. Karakiewicz, Quoc-Dien Trinh, Vincenzo Ficarra et al. "Prognostic ability of simplified nuclear grading of renal cell carcinoma", Cancer, 2007 Publicaţie	<1 %
54	ouci.dntb.gov.ua Sursă de pe Internet	<1 %
55	www.mysciencework.com Sursă de pe Internet	<1 %
56	Giacomo Rebez, Nicola Pavan, M. Carmen Mir. "Available active surveillance follow-up protocols for small renal mass: a systematic review", World Journal of Urology, 2021 Publicaţie	<1 %
57	James J. Hsieh, Mark P. Purdue, Sabina Signoretti, Charles Swanton et al. "Renal cell carcinoma", Nature Reviews Disease Primers, 2017 Publicaţie	<1 %
58	Lane, Brian R., Conrad M. Tobert, and Christopher B. Riedinger. "Growth kinetics	<1 %

and active surveillance for small renal masses :", Current Opinion in Urology, 2012.
Publicaţie

59	discovery.dundee.ac.uk Sursă de pe Internet	<1 %
60	synapse.koreamed.org Sursă de pe Internet	<1 %
61	www.endocrine-abstracts.org Sursă de pe Internet	<1 %
62	www.minervamedica.it Sursă de pe Internet	<1 %
63	"The Management of Small Renal Masses", Springer Science and Business Media LLC, 2018 Publicaţie	<1 %
64	Raimonda Kubiliute, Algirdas Zalimas, Arnas Bakavicius, Albertas Ulys, Feliksas Jankevicius, Sonata Jarmalaite. "Clinical Significance of ADAMTS19, BMP7, SIM1, and SFRP1 Promoter Methylation in Renal Clear Cell Carcinoma", OncoTargets and Therapy, 2021 Publicaţie	<1 %

Excludeţi citările Dezactivat
Excludeţi bibliografia Dezactivat
Excludeţi similitudinile Dezactivat