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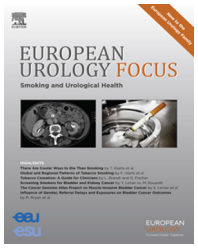

Review – Benign Prostatic Hyperplasia

The Potential Role of MicroRNAs as Biomarkers in Benign Prostatic Hyperplasia: A Systematic Review and Meta-analysis

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Abstract

Context: Benign prostate hyperplasia (BPH) is one of the most common urologic diseases. However, the molecular and cellular mechanisms involving the stromal and epithelial components of the prostate that lead to BPH remain unclear.
Objective: To review and evaluate the evidence implicating microRNAs (miRNAs) in the pathogenesis of BPH.
Evidence acquisition: A systematic search of the PubMed and Embase databases was performed using the terms "benign prostate hypertrophy and miRNA" or ("benign prostate hypertrophy and microRNAs" or "miRNA" or "miR") on July 31, 2017.
Evidence synthesis: Sixty-four miRNAs from 37 selected articles were ranked according to p values ($p \leq 0.05$). To avoid false positive results, Benjamini-Hochberg correction of p values was performed. Application of the robust rank aggregation method identified miR-221 as significantly associated with BPH ($p = 0.013$). The effect size (ES) was calculated for studies with miR-221 data to generate an estimate of the overall ES and its confidence interval. The ES for miR-221 was measured by the standardized mean difference obtained by dividing the difference in the average gene expression between the PCa and BPH groups by a pooled estimate of standard deviation. The random effects model was used to calculate the pooled ES due to the presence of heterogeneity among studies. Publication bias of the seven included studies was assessed by the Funnel plot and Egger's test and it was detected in the overall analysis of the seven studies ($p < 0.01$). After the trim and fill procedure, Egger's test revealed no evidence of publication bias ($p = 0.76$)
Conclusions: miR-221 has the potential to be used both as a biomarker and novel target in the early diagnosis and therapy of BPH. Technological advances should enable the synthesis of pre-RNA or anti-RNA molecules within carrier vehicles that can be safely delivered into patients. The development of such new pharmacologic therapies should be lastly investigated as possible therapy of one of the most common urologic diseases among elderly men.
Patient summary: miR-221 has the potential to be used both as a biomarker and novel target in the early diagnosis and therapy of benign prostate hyperplasia. The development of new pharmacologic therapies enabling the synthesis of anti-miR-221 should be lastly investigated as a possible therapy of one of the most common urologic diseases among elderly men.

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1. Introduction

Benign prostatic hypertrophy (BPH) represents one of the most common urologic diseases among elderly men with a major impact on quality of life (QoL) and a substantial economic burden [1,2].

Epidemiologic studies suggest that age, genetic factors, and sexual hormones play major roles as risk factors for BPH, and the only known factors associated with BPH progression are age and prostate volume [3]. More recently, metabolic syndrome, detrusor overactivity, prostatic inflammation, cell-signaling disorders, and neurologic, cardiac, and renal dysfunctions have been hypothesized to contribute to the development of BPH [4]. Despite the well-established role of age, hormonal factors, and genetic predisposition, the molecular and cellular mechanisms involving the stromal and epithelial components of the prostate that lead to BPH remain unclear [5]. In the last few years it has been postulated an influence of microRNAs (miRNAs) in the regulation of urogenital diseases [6]. miRNAs are a group of small RNA molecules of between 19 and 25 nucleotides that can target many genes, as well as genes within the same pathway, making them central regulatory nodes. Deregulated miRNAs can contribute to carcinogenesis as oncomirs, which regulate tumor suppressors, or as tumor suppressor miRNAs, which target oncogenes [7].

In 2011, a systematic review by Catto et al [6] evaluated the association of miRNAs with prostate, kidney, and bladder cancers, concluding that miRNAs are frequently altered in urologic cancers and as such have the potential to serve as biomarkers or novel therapeutic targets.

Aberrant miRNA expression and specific miRNA signatures have been identified by many groups, demonstrating the involvement of miRNAs in the pathophysiology of prostate cancer (PCa) [8–10].

A recent study identified miR-19a, miR-32, miR-124a, miR-130b, miR-148a, and miR-583 as potential regulators of *FLNC*, *MSRB3*, *PARVA*, *PCDH7*, *PRNP*, *RAB34*, and *SORBS1*, which correlate with prostate-specific antigen-relapse in PCa patients [10]. Furthermore, several deregulated miRNAs were identified by different groups using different methodologies, offering encouraging results about the prospective use of miRNAs in the diagnosis, prognosis, and therapy of PCa patients [9].

Accumulating evidence supports pathologic links between BPH and PCa in addition to the well-established epidemiologic associations. Anatomic and genetic data also suggest that many PCas are associated with preexisting BPH [10].

The rate of BPH growth is gaining increasing support as both a predictive and prognostic factor for PCa: fast-growing BPH is linked to an increased risk of developing PCa and an increased likelihood that such cancer will be of a high stage or grade [11].

However, knowledge about the association between miRNAs and BPH remains limited, despite the fact that a biomarker for the early diagnosis of BPH could offer the potential to optimize the prevention and treatment of these two commonly coexisting diseases.

The aim of this systematic review was to evaluate the evidence implicating miRNAs in the pathogenesis of BPH.

2. Evidence acquisition

A detailed literature search of the Science Direct and PubMed repositories was performed using the strings "benign prostate hypertrophy and miRNA" or ("benign prostate hypertrophy and microRNAs" or "miRNA" or "miR") on July 31, 2017.

Relevant papers were selected by two authors (F.G. and S. G.) and all authors fine-tuned and enhanced the list of papers to be included.

Review articles, editorials, commentaries, and letters to the editor were included only if deemed to contain relevant information. Cited references from the selected articles and from review articles retrieved in the search were assessed for significant manuscripts not previously included. Studies published only as an abstract or presented without an abstract, and reports from meetings and studies not published in English were not included in the review. Studies focused on prognosis of PCa, cell line-based studies, and studies with unqualified data were also excluded from the analysis.

The authors selected 37 articles according to the search strategy based on Preferred Reporting Items for Systematic Reviews and Meta-analyses criteria (Fig. 1, Table 1) [12].

Results were frequently reported in a list of miRNAs up- or down-regulated with p values as a measure of significance and fold change indicating expression differences between the disease and control groups.

The most frequently used approach to the analysis of miRNAs and the meta-analysis was based on the Robust Rank Aggregation (RRA) method [13,14].

In brief, the method consisted of a comparison of several commonly overlapping miRNAs in a ranked list arising from different studies. Rendering miRNA names comparable across studies was an essential step to achieve a comprehensive meta-analysis.

The selected miRNAs, previously identified in the included studies, were examined by performing a meta-analysis using the Random Effects Model in *metafor* R-package (R-foundation, Vienna, Austria) [14].

3. Evidence synthesis

The initial search returned a total of 346 manuscripts from the PubMed (n = 68) and Science Direct (n = 278) databases, of which 271 were excluded because of duplication among databases or because they were posters, abstracts, or not Homosapien related. Finally, 37 articles including 1561 BPH patients from several countries were subjected to further full-text review (Table 1).

Thirty-five studies examined the expression of miRNAs by quantitative real-time reverse transcription PCR (qRT-PCR) in samples of tissue (n = 20) [8,15–33], urine (n = 2) [34,35], serum (n = 2) [36,37], blood (n = 10) [38–47], or in all three specimens (n = 1) [48]; only two studies performed

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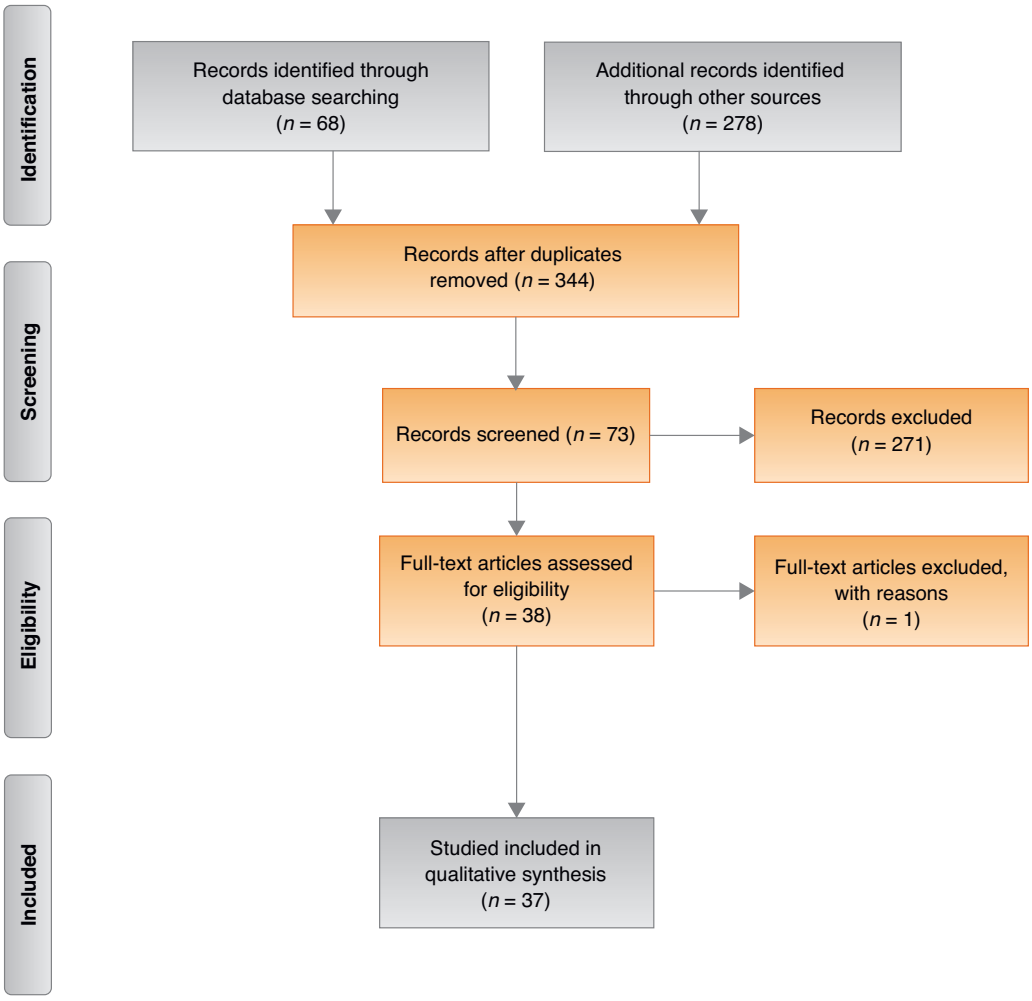

Fig. 1 – Preferred Reporting Items for Systematic Reviews and Meta-analyses flow diagram.

high throughput sequencing to compare PCa and BPH using plasma ($n = 1$) [49] and tissue ($n = 1$) [50] samples.

The information obtained from multiple independent studies from different platforms was combined in two fundamental steps to perform the meta-analysis.

3.1. *miRNA selection*

In the first step, 64 miRNAs from selected articles were ranked according to p values ($p \leq 0.05$). The ranked lists of miRNAs from each study were combined into a single gene ranking and the RRA method was applied. To avoid false positive results, the Benjamini-Hochberg correction was calculated to adjust p values. After using the RRA method, miR-221 was identified as significantly associated with BPH ($p = 0.013$).

In 2015, Gunzel et al [27] aimed to identify a miRNA expression signature that could be used to distinguish PCa from BPH. Microarray miRNA profiling of four PCa and four BPH patients revealed that miR-361-3p, miR-133b, and miR-221 were significantly downregulated and miR-203 was upregulated in prostate secretion samples of PCa patients. qRT-PCR results demonstrated that the expression of miR-361-3p was significantly lower in the prostate secretion samples of PCa patients than in those of patients with BPH ($p = 0.004$). mir-133b (Fig. 2; $p < 0.01$) and mir-221 ($p = 0.03$) were also downregulated in PCa compared with BPH. By contrast, the expression levels of mir-203 were significantly upregulated in PCa samples with a p value of 0.0002.

Similarly, Leideinger et al [43] used qRT-PCR to validate five miRNAs that were deregulated between PCa and BPH patients in the microarray analysis. The selected miRNAs included miR-708* and miR-221*, which were downregulated in PCa, and miR-675, miR-1180, and miR-659, which were upregulated in PCa compared with BPH. The significance values by unpaired t test on the Cq values were 0.03 for miR-1180, 0.58 for miR-221*, 0.07 for miR- 659, 0.046 for miR-675, and 0.19 for miR-708*. The direction of regulation was confirmed for hsa-miR-708* and hsa-miR-221*. For both miRNAs, the extent of the downregulation was lower by qRT-PCR than by microarray analysis: hsa-miR-708* was downregulated with a fold change of 2.3 and 1.2 and hsa-miR-221* with a fold change of 3.3 and 1.1 by microarray and qRT-PCR analysis, respectively.

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Table 1 – Selected studies.

Reference	Count	Yr	Country	Ethnicity	Sample size (*n*)	Mean age (yr)	miRNAs	Specimen	Technology	Oxford LoE	miR-221
15	1	2010	Sweden	Caucasian	25	71	miR-34c	Tissue	q-PCR	A	–
16	1	2010	Czech Republic	Caucasian	53	N.R.	mir-20a (2), let-7a (3), miR-15a (2), miR-16 (4)	Tissue	q-PCR	B	–
36	6	2011	Germany	Caucasian	18	N.R.	miR-16, miR-26a (2), miR-32 (3), miR-195, miR-39, miR-7i	Serum	q-PCR	B	–
17	1	2011	USA	Caucasian	25	N.R.	miR-145 (5)	Tissue	q-PCR	B	–
49	1146	2012	China	Asian	44	72	miR-30c (3), miR-622, miR-1285, miR-7c, miR-7e	Plasma	q-PCR-HTS	A	–
18	723	2012	Finland	Caucasian	12	N.R.	miR-21 (6), miR-32, miR-99a, miR-99b, miR-148a, miR-221 (4), miR-590-5p	Tissue	q-PCR	B	*p* value = 0.00280, CP = 42
19	1	2013	Greece	Caucasian	66	70	miR-244	Tissue	q-PCR	A	–
20	2	2013	Brasil	Caucasian	44	66	miR-143 (2), miR-145	Tissue	q-PCR	A	–
21	1002	2013	China	Asian	3	N.R.	miR-182, miR-221, miR-320e, miR-320b, miR-145, miR27b, miR-378, miR-4306, miR-3162, miR-3147, miR-3156, miR-664, miR-4271, miR-625, miR-3652, miR-1306, miR-3714, miR-4323, miR-345, miR-1182, miR-4257, miR-3675-3p, miR-3149, miR-15b, miR-936, miR-4270	Tissue	q-PCR	B	*p* value = 2.60E-02, CP = 5
22	1	2013	Greece	Caucasian	64	N.R.	miR-145	Tissue	q-PCR	B	–
34	894	2014	USA	Caucasian	12	N.R.	miR-1825, miR-484	Urine	q-PCR	B	–
23	1	2014	Germany	Caucasian	15	66	miR-203	Tissue	q-PCR	A	–
24	9	2014	Germany	Caucasian	30	72	miR-101 (2), miR-138, miR-186, miR-224, miR-26a, miR-26b (2), miR-374a, miR-410, miR-660	Tissue	q-PCR	A	–
25	1	2015	Finland	Caucasian	10	62	miR-193b	Tissue	q-PCR	A	–
38	3	2015	Serbia	Caucasian	353	N.R.	miR-499, miR-196a2, miR-27a	blood	q-PCR	B	–
35	2	2015	Italy	Caucasian	26	66,5	miR-191 (2), miR-25 (2)	urine	q-PCR	A	–
26	3	2015	Kolkata	Caucasian	25	69	miR-21, miR-155, miR-141 (4)	Tissue	q-PCR	A	–
37	21	2015	USA	Caucasian	50	66	miR-1274a, miR-141, miR182, miR183, miR-96, let-7a, miR-103, miR-107, miR-130b, miR-106a, miR-26b, miR-451, miR-223, miR-93, miR-24, miR-30c, miR-874, miR-100, miR-146a, miR-125b, miR-1207-5b	Serum	q-PCR	A	–
39	1	2015	Germany	Caucasian	35	50–89	miR-375 (3)	Blood	q-PCR	A	–
50	2042	2015	China	Asian	24	69	miR-125b-5p, miR-126-5p, miR-139-3p, miR-151a-5p, miR-151b, miR-193a-5p, miR-206, miR-221-3p, miR-222-3p, miR-365b-3p, miR-6087, miR-708-3p, miR-95-3p, miR-144-3p, miR-451a, miR-486-5p, miR-655-3p, miR-887.	Tissue	HTS-qPCR	A	–
40	4	2015	Bulgaria	Caucasian	16	66	let-7c, miR-30c, miR-141, miR-375	Blood	q-PCR	A	–
27	470	2015	Turkey	Caucasian	25	63.5	miR-361-3p, miR-221, miR-133b, miR-203	Tissue	q-PCR	A	*p* value = 0.03, CP = 23
28	1	2016	Bahrain	Caucasian	24	70	miR-18a	Tissue	q-PCR	B	–
41	9	2014	Denmark	Caucasian	13	70.5	miR-17, miR-200b, miR-210, miR-297, miR-375, miR-501-3p, miR-551b, miR-562, let-7a	Blood	q-PCR	B	–
29	6	2011	China	Asian	12	68	miR-21, miR-125b, miR-101, miR-141, miR-16, miR34c	Tissue	q-PCR	A	–
42	4	2016	Iran	Caucasian	182	62.5	miR-146a, miR-149, miR-196a2, miR-499 polymorphisms	Blood	T-ARMS-PCR, PCR-RFLP	B	–
43	864	2016	Germany	Caucasian	39	67.6	864 miRNAs (miR-708, miR-221, miR-518d-5p, miR-675, miR-1180, miR-1225-5p, and miR-659)	Blood	q-PCR	A	*p* value = 0.0008, CP = 76
30	14	2011	N.R.	N.R.	10	68.5	let7c, miR-15a, miR-16, miR-21, miR-25, miR-32, miR-100, miR-143, miR-145, miR-146a, miR-191, miR-199a, miR-206, miR-218	Tissue	q-PCR	A	–

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Table 1 (*Continued*)

Reference	Count	Yr	Country	Ethnicity	Sample size (*n*)	Mean age (yr)	miRNAs	Specimen	Technology	Oxford LoE	miR-221
44	1	2016	China	Asian	45	69	miR-139-5p	Blood	q-PCR	A	–
8	319	2007	USA	N.R.	4	N.R.	319 miRNAs (let-7a, let-7f, miR-202, miR-184, let-7b, miR-19b, miR-210, miR-198, let-7c, miR-22, miR-296, miR-302c*,let-7d, miR-26b, miR-320, miR-345, let-7g, miR-27a, miR-370, miR-491, miR-16, miR-27b, miR-373*, miR-513, miR-23a, miR-29a, miR-498, miR-23b, miR-29b, miR-503, miR-26a, miR-30a-5p, miR-92, miR-30b, miR-99a, miR-30c, miR-103, miR-100, miR-125a, miR-141, miR-125b, miR-148a, miR-143, miR-205, miR-145, miR-195, miR-199a, miR-221, miR-222, miR-497)	Tissue	q-PCR	B	*p* value = 0.0028, CP = 9
31	254	2010	Finland	Caucasian	4	N.R.	254 microRNAs, miR-193b	Tissue	q-PCR	B	*p* value = 0.005, CP = 9
45	4	2016	Spain	Caucasian	31	66.7	miR-106a, miR-20a, miR-223, miR-21	Blood	q-PCR	A	–
46	1	2016	China	Asian	81	N.R.	miR-410-5p	Blood	q-PCR	A	–
48	4	2015	Korea	Asian	N.R.	N.R.	miR-615-3p, hsv1-miR-H18, hsv2-miR-H9-5p, miR-4316	Urine, blood, tissue	q-PCR	A	–
32	2	2016	Korea	Asian	131	71,5	hsv1-miR-H18, hsv2-miRH9-5p	Tissue	q-PCR	A	–
47	1	2011	China	Asian	6	68.9	miR-21	Blood	q-PCR	A	–
32	665	2010	Germany	Caucasian	4	N.R.	665 miRNAs (miR-10441, miR-2660, miR-9534, miR-7425, miR-363, miR-207, miR-3896, miR-291b, miR-138, miR-449, miR-485, miR-9651, miR-9508, miR-7, miR-190, miR-122a, miR-9673, miR-9125, miR-205, miR-181a, miR-22, miR-23b, miR-221, miR99a, miR-24, miR-17, miR-16, miR-342, miR-320, miR-21, miR-34a, miR-30a, miR-15a, miR-140, miR-125b, miR-27b, miR-29a, miR-130a, miR-199a, miR-99a, miR-30e, miR-145, miR-125a, miR-422b, miR-100, miR-143, miR-222, miR-92, miR-497, miR-193a, miR-miR-23a, miR-194, miR-195, mIR-192, let-7c, miR-150, miR-125, miR193b, miR106b, miR-214, miR133a, miR-30b, miR-13193)	Tissue	q-PCR	B	*p* value = 0.01, CP = 8

HTS = high throughput sequencing; LoE = level of evidence; miRNA = microRNA; N.R. = not reported; q-PCR = quantitative polymerase chain reaction; RFLP = restriction fragment length polymorphism.

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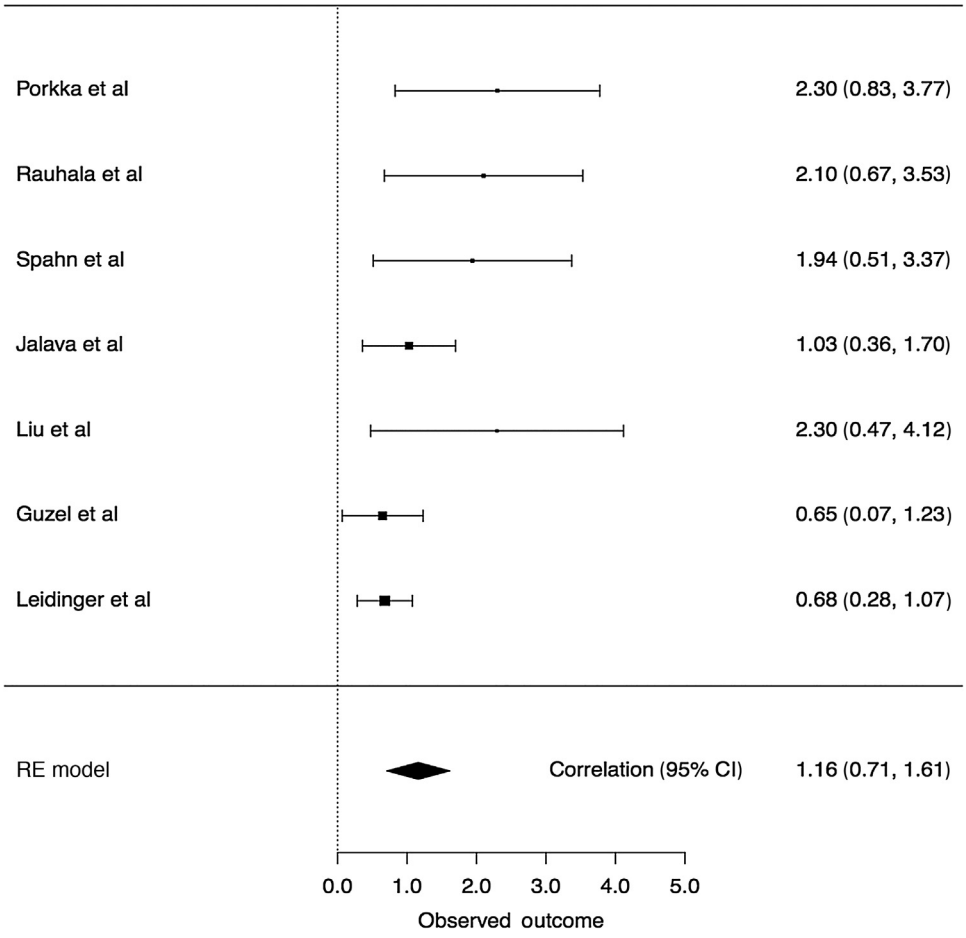

Fig. 2 – Forest plot investigating the relationship between benign prostate hyperplasia and microRNA-221.
CI = confidence interval; RE = random effects.

3.2. *Analysis of the correlation between BPH and miR-221*

The effect size (ES) was combined only for studies containing miR-221 data (Table 2) to generate an estimate of the overall ES and its confidence interval. Choi et al [51] described a method to combine ES using a random-effects modeling approach to merge datasets from individual studies of two groups, obtaining an overall estimate of the weighted ES. The ES for miR-221 was measured by the standardized mean difference obtained by dividing the difference in the average gene expression between the PCa and BPH groups by a pooled estimate of standard deviation. The ES was used to measure the magnitude of the treatment effect in each study, and a random effects model was used to incorporate interstudy variability [52].

Figure 2 shows the forest plot of the analysis of miR-221. The random effects model was used to calculate the pooled ES because of the presence of heterogeneity among the studies ($I^2 = 46.2\%$, $p = 0.05$, 95% confidence interval: 0.71–1.61), which could be attributed to the wide-ranging origins of the study cohorts. The studies by Porkka et al [8] and Rauhala et al [31] were based on cohorts of four patients with BPH and nine with PCa; Spahn et al [33] reported only four BPH and eight PCA cases, and Liu et al [21] also had a

Table 2 – Overall effect size and its confidence interval for studies with miR-221 data.

Reference	Yr	ES	Weight	Sample size	SE	Var	CI (low)	CI (high)
8	2007	2.300847844	1.770780659	13	0.751480326	0.56472268	0.827973459	3.77372223
31	2010	2.101203615	1.883519459	13	0.728643247	0.530920982	0.673089082	3.529318149
33	2010	1.940775226	1.87990413	12	0.729343554	0.53194202	0.511288116	3.370262336
18	2012	1.027188165	8.553409322	54	0.341924619	0.116912445	0.357028221	1.69734811
21	2013	2.2950805	1.159359143	8	0.928733272	0.86254549	0.474796722	4.115364278
27	2015	0.647048261	11.38441009	48	0.296377161	0.087839422	0.066159694	1.227936827
43	2016	0.678747569	24.50862877	115	0.201994941	0.040801956	0.282844756	1.074650382

CI = confidence interval; ES = effect size; SE = standard error; Var = variant.

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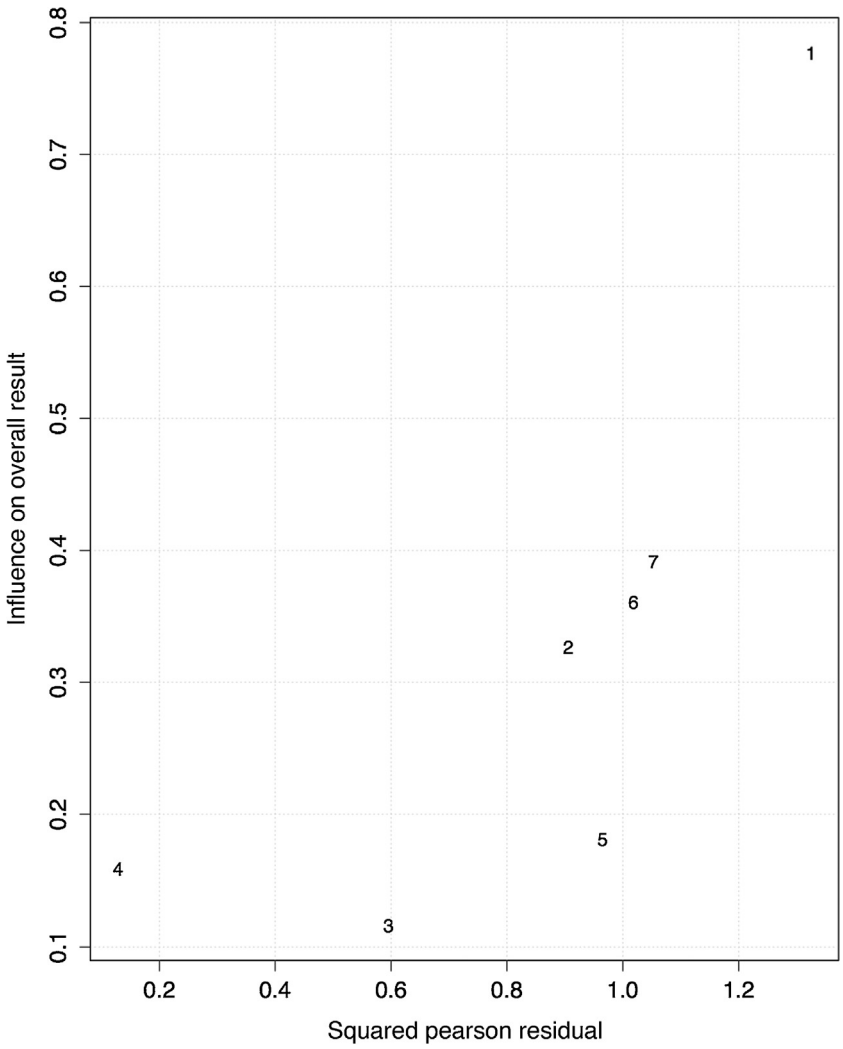

Fig. 3 – Baujat plot shows the contribution to heterogeneity for each study represented by *Id* number.

limited cohort size (3 BPH and 5 PCa patients). Higher numbers of patients were reported by Jalava et al [18] (12 BPH and 42 PCa), Guzel at al [27] (25 BPH and 23 PCa), and Leidinger et al [43] (39 BPH and 76 PCa).

To identify studies that may disproportionately influence heterogeneity, Baujat et al [53] in 2002 proposed a plot to identify studies contributing excessively to heterogeneity and the overall result. The Baujat plot (Fig. 3) identified study 1, Porkka et al [8], as contributing excessively to heterogeneity and the overall result. A closer look at the characteristics of this study revealed considerable differences in sample size compared with others included in the meta-analysis.

The Funnel plot and Egger's test were used to assess potential publication bias in the seven included studies (Fig. 4). Publication bias was detected in the overall analysis of the seven studies ($p < 0.01$). However, after the trim and fill procedure [54], Egger's test revealed no evidence of publication bias ($p = 0.76$) as show in Fig. 5. This method adjusts a meta-analysis by imputing "missing" studies to increase funnel plot symmetry. Use of the trim and fill method to adjust the current data provided a reasonable estimate of three missing studies.

4. Conclusions

BPH is a unique condition that is no longer life-threatening; however, its potentially progressive nature has a negative impact on men's QoL, daily activities, and general health, which may determine the need for lifelong medical therapy or surgery [55]. Moreover, this pathology also presents important social consequences. Its burden extends beyond the sufferer; partners of men with BPH also have significant morbidity associated with their husband's condition [56].

In addition to the clinical and social implications of the pharmacological and surgical treatment of BPH, the economic costs associated with this disease need to be considered.

In the UK, it is estimated that more than £180 million is spent on BPH treatments each year, with 60% of these costs incurred in secondary care as a direct result of managing BPH complications [57].

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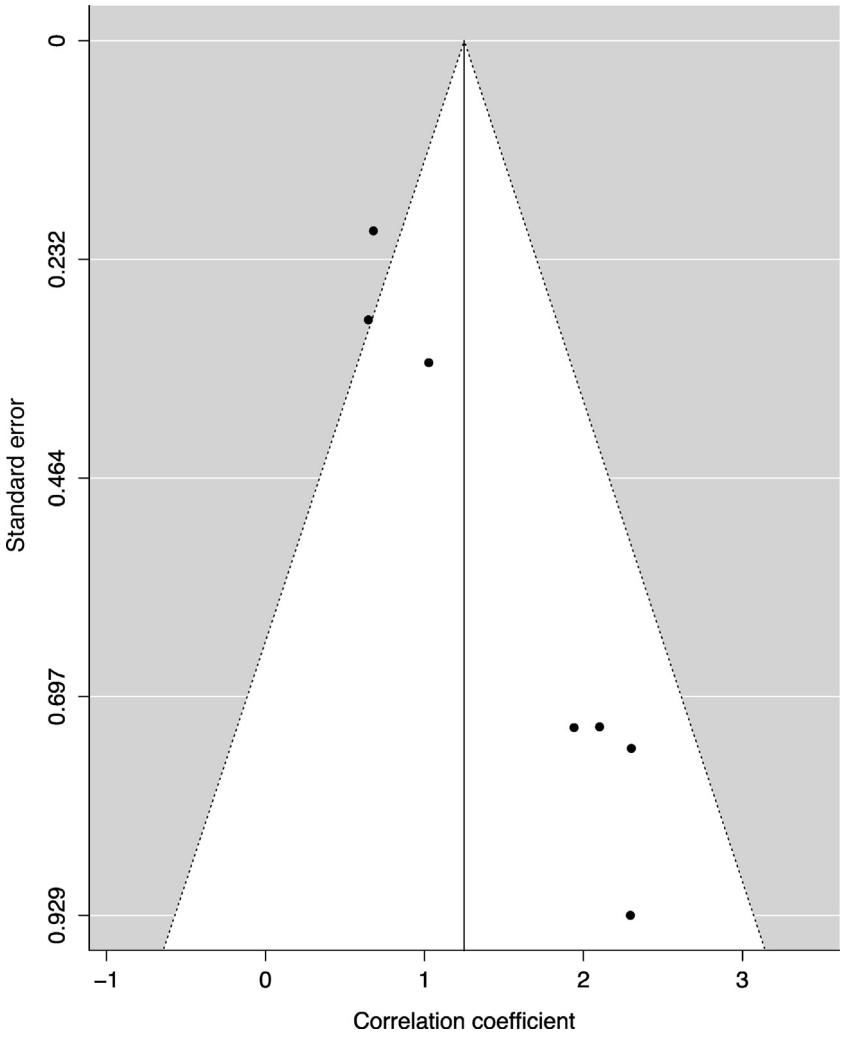

Fig. 4 – Funnel plot shows the publication bias for all studies in meta-analysis. Rank correction ($p = 0.03$) and regression ($p = 0.0006$) test were statistically significant, consistent with asymmetry, and potential publication bias.

In the prospective, cross-sectional, observational Trans European Research into the use of Management Policies for Lower Urinary Tract Symptoms suggestive of BPH in Primary Healthcare survey, the mean total 1-yr treatment costs were €858/patient affected by BPH, ranging from €292 in the UK to €1337 in Poland. Medication was by far the most important cost driver, as it accounted for approximately three quarters of the total treatment costs. Surgery costs amounted to 15% and diagnostic testing to 8% of the total cost [58].

All these clinical, social, and economic problems related to the progressive nature of BPH could be resolved by improving our understanding of the molecular and cellular mechanisms involving the stromal and epithelial components of the prostate that lead to BPH.

In recent years, there has been an increasing interest in the codification of miRNAs for the development of therapies for urological diseases.

Because each miRNA can modulate the expression of multiple messengerRNAs (mRNAs) and each mRNA may be targeted by several different miRNAs, it is not surprising that miRNAs are involved in almost all key cellular processes, such as proliferation, differentiation, migration, apoptosis, and stemness maintenance [9].

A growing body of literature has investigated the potential use of miRNAs as biomarkers for cancer diagnosis, prognosis, and therapy [59], including in PCa [60], and the association of miRNA expression levels with clinicopathologic parameters has been demonstrated [50,61,62]. The expression levels of specific miRNAs in clinical prostate tissue samples may be used to detect cancer, predict cancer prognosis, and monitor its evolution, and as markers for therapy selection and response [9].

Accumulating evidence supports that many prostate cancers are associated with preexisting BPH. The rate of BPH growth is gaining increasing support as both a predictive and prognostic factor for PCa: fast-growing BPH is linked with an increased risk of developing PCa and an increased likelihood that such cancer will be of a high stage or grade [11].

Considering the pathologic links between BPH and PCa, we hypothesized that miRNAs expressed in PCa could also be present in patients affected by BPH.

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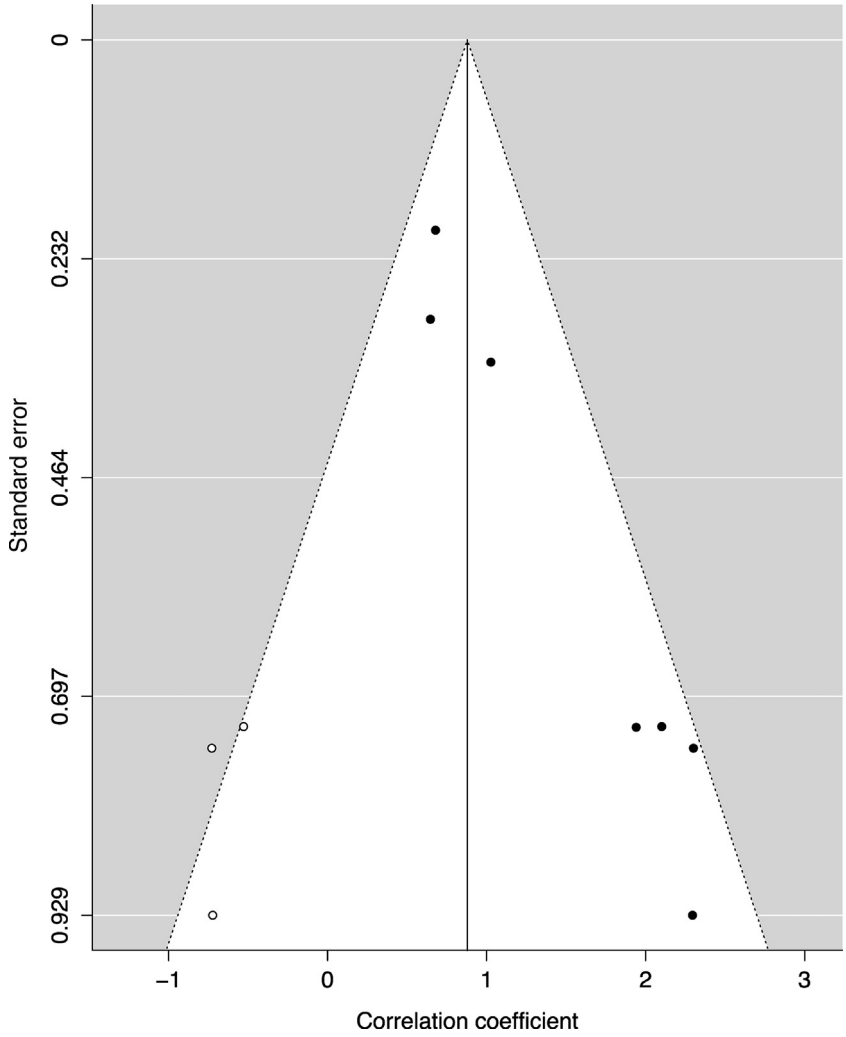

Fig. 5 – Trim and Fill procedure imputes missing values (hallow circles) to create a more symmetrical funnel plot.

We performed a systematic review of the literature and considered all studies investigating miRNAs in PCa that included patients affected by BPH as a control group.

Ranked lists of miRNAs produced for each study were aggregated into a single gene ranking and analyzed using the RRA method, which confirmed miR-221 as the only miRNA significantly associated with BPH ($p = 0.013$). Only seven studies reported data for miR-221. Publication bias in the seven included studies was assessed by Funnel plot and Egger's test. Publication bias was detected in the overall analysis of seven enrolled studies ($p < 0.01$). However, after the trim and fill procedure [54], Egger's test revealed no evidence of publication bias ($p = 0.76$).

Jalava et al [18] identified five miRNAs regulated by dihydrotestosterone, which plays a beneficial role in the developing prostate [63]: miR-32, miR-148a, miR-99a, miR-21, and miR-221. Four of these, namely miR-32, miR-590-5p, miR-148a, and miR-21 were significantly overexpressed, and three, miR-99a, miR-99b, and miR-221, were significantly underexpressed in castration-resistant PCa.

Liu et al [21] reported that miR-182 expression was significantly upregulated in PCa tissues and in four cell lines compared with BPH, whereas five miRNAs (miR-345, miR-145, miR-221, miR-27b, and miR-378) were downregulated in PCa.

Furtermore, in all studies evaluating the correlation between miRNA-221 and PCa, miRNA-221 was down-regulated in patients with higher Gleason scores, advanced stage tumors, and positive lymph nodes [8,18,21,27,31,33,43].

Recently, Gheinani et al [64] reported mRNA and miRNA transcriptome sequencing of bladder samples from human patients with different urodynamically defined states of bladder outlet obstruction (BOO). From 19 upregulated and 17 downregulated BOO miRNAs, only 221 and 215 mRNA targets, respectively, were present in the BOO mRNA data set.

Although our meta-analysis highlights the potential value of miR-221 as a potential biomarker for BPH, with diagnostic and treatment implications, we cannot forget that BPH is a multifactorial disease, whose pathophysiology has still not been completely elucidated. Another limitation is represented by the lack of studies including as control group patients with PCa or age-matched asymptomatic men with no lower urinary tract symptoms.

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In the future, the technology will become less expensive and more prevalent, and it is likely that further advances in our knowledge of the role of miRNAs in human disease will be made. With respect to urologic malignant and benign diseases, miRNAs hold promise as potent biomarkers and novel therapeutic targets. Clinical trials using RNA therapies are awaited, and it is likely that within the next few years these trials will affect the way we treat our patients [6,65].

miRNAs are important modulators of gene expression. They are frequently altered in urologic cancers and they play an important role in the pathogenesis of BPH.

miR-221 has the potential to be used both as a biomarker and novel target in the early diagnosis and therapy of BPH. Technological advances should enable the synthesis of pre-RNA or anti-RNA molecules within carrier vehicles that can be safely delivered into patients. These molecules could be administered topically or systematically to induce generalized cell targeting.

The development of such new pharmacologic therapies should be lastly investigated as possible therapy of one of the most common urologic diseases among elderly men with a major impact on QoL, in an attempt to reduce the great economic burden associated with this disease.

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Study concept and design: Greco, Gangemi.
Acquisition of data: Greco, Inferrera, La Rocca, Casciaro.
Analysis and interpretation of data: Greco, Navarra, Gangemi.
Drafting of the manuscript: Greco.
Critical revision of the manuscript for important intellectual content: Ficarra, Mirone.
Statistical analysis: Greco, Gangemi.
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References

[1] Cartwright R, Mangera A, Tikkinen KA, et al. Systematic review and meta-analysis of candidate gene association studies of lower urinary tract symptoms in men. Eur Urol 2014;66:752–68.
[2] Parsons JK, Messer K, White M, et al. Obesity increases and physical activity decreases lower urinary tract symptom risk in older men: The Osteoporotic Fractures in Men study. Eur Urol 2011;60:1173–80.
[3] De Nunzio C, Ahyai S, Autorino R, et al. Benign prostatic hyperplasia and lower urinary tract symptoms: research priorities. Eur Urol 2011;60:1205–6.
[4] Maserejian NN, Giovannucci EL, McKinlay JB. Dietary macronutrients, cholesterol, and sodium and lower urinary tract symptoms in men. Eur Urol 2009;55:1179–89.
[5] Tang J, Yang J. Etiopathogenesis of benign prostatic hyperplasia. Indian J Urol 2009;25:312–7.
[6] Catto JW, Alcaraz A, Bjartell AS, et al. MicroRNA in prostate, bladder, and kidney cancer: a systematic review. Eur Urol 2011;59:671–81.
[7] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009;10:704–14.
[8] Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T. MicroRNA expression profiling in prostate cancer. Cancer Res 2007;67:6130–5.
[9] Fabris L, Ceder Y, Chinnaiyan AM, et al. The potential of microRNAs as prostate cancer biomarkers. Eur Urol 2016;70:312–22.
[10] Aakula A, Kohonen P, Leivonen S, et al. Systematic identification of micrornas that impact on proliferation of prostate cancer cells and display changed expression in tumor tissue. Eur Urol 2016;69:1120–8.
[11] Alcaraz A, Hammerer P, Tubaro A, et al. Is there evidence of a relationship between benign prostatic hyperplasia and prostate cancer? Findings of a literature review. Eur Urol 2009;55:864–75.
[12] Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 2015;4:1.
[13] Võsa U, Vooder T, Kolde R, Vilo J, Metspalu A, Annilo T. Meta-analysis of microRNA expression in lung cancer. Int J Cancer 2013;132:2884–93.
[14] Kolde R, Laur S, Adler P, Vilo J. Robust rank aggregation for gene list integration and meta-analysis. Bioinformatics 2012;28:573–80.
[15] Hagman Z, Larne O, Edsjö A, et al. miR-34c is downregulated in prostate cancer and exerts tumor suppressive functions. Int J Cancer 2010;127:2768–76.
[16] Pesta M, Klecka J, Kulda V, et al. Importance of miR-20a expression in prostate cancer tissue. Anticancer Res 2010;30:3579–83.
[17] Suh SO, Chen Y, Zaman MS, et al. MicroRNA-145 is regulated by DNA methylation and p53 gene mutation in prostate cancer. Carcinogenesis 2011;32:772–8.
[18] Jalava SE, Urbanucci A, Latonen L, et al. Androgen-regulated miR-32 targets BTG2 and is overexpressed in castration-resistant prostate cancer. Oncogene 2012;31:4460–71.
[19] Mavridis K, Stravodimos K, Scorilas A. Downregulation and prognostic performance of microRNA 224 expression in prostate cancer. Clin Chem 2013;59:261–9.
[20] Viana NI, Reis ST, Dip NG, et al. MicroRNAs 143 and 145 may be involved in benign prostatic hyperplasia pathogenesis through regulation of target genes and proteins. Int J Biol Markers 2014;29:e246–52.
[21] Liu R, Li J, Teng Z, Zhang Z, Xu Y. Overexpressed microRNA-182 promotes proliferation and invasion in prostate cancer PC-3 cells by down-regulating N-myc downstream regulated gene 1 (*NDRG1*). PLoS One 2013;8:e68982.
[22] Avgeris M, Stravodimos K, Fragoulis EG, Scorilas A. The loss of the tumour-suppressor miR-145 results in the shorter disease-free survival of prostate cancer patients. Br J Cancer 2013;108:2573–81.
[23] Hailer A, Grunewald TG, Orth M, et al. Loss of tumor suppressor mir-203 mediates overexpression of LIM and SH3 Protein 1 (LASP1) in high-risk prostate cancer thereby increasing cell proliferation and migration. Oncotarget 2014;5:4144–53.
[24] Erdmann K, Kaulke K, Thomae C, et al. Elevated expression of prostate cancer-associated genes is linked to down-regulation of microRNAs. BMC Cancer 2014;14:82.
[25] Kaukoniemi KM, Rauhala HE, Scaravilli M, et al. Epigenetically altered miR-193b targets cyclin D1 in prostate cancer. Cancer Med 2015;4:1417–25.

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[26] Basu S, Majumder S, Bhowal A, et al. A study of molecular signals deregulating mismatch repair genes in prostate cancer compared to benign prostatic hyperplasia. PLoS One 2015;10:e0125560.
[27] Guzel E, Karatas OF, Semercioz A, et al. Identification of microRNAs differentially expressed in prostatic secretions of patients with prostate cancer. Int J Cancer 2015;136:875–9.
[28] Al-Kafaji G, Al-Naieb ZT, Bakhiet M. Increased oncogenic microRNA-18a expression in the peripheral blood of patients with prostate cancer: A potential novel non-invasive biomarker. Oncol Lett 2016;11:1201–6.
[29] Hao Y, Zhao Y, Zhao X, et al. Improvement of prostate cancer detection by integrating the PSA test with miRNA expression profiling. Cancer Invest 2011;29:318–24.
[30] Leite KR, Tomiyama A, Reis ST, et al. MicroRNA-100 expression is independently related to biochemical recurrence of prostate cancer. J Urol 2011;185:1118–22.
[31] Rauhala HE, Jalava SE, Isotalo J, et al. miR-193b is an epigenetically regulated putative tumor suppressor in prostate cancer. Int J Cancer 2010;127:1363–72.
[32] Yun SJ, Jeong P, Kang HW, et al. Increased expression of herpes virus-encoded hsv1-miR-H18 and hsv2-miR-H9-5p in cancer-containing prostate tissue compared to that in benign prostate hyperplasia tissue. Int Neurourol J 2016;20:122–30.
[33] Spahn M, Kneitz S, Scholz CJ, et al. Expression of microRNA-221 is progressively reduced in aggressive prostate cancer and metastasis and predicts clinical recurrence. Int J Cancer 2010;127:394–403.
[34] Haj-Ahmad TA, Abdalla MA, Haj-Ahmad Y. Potential urinary miRNA biomarker candidates for the accurate detection of prostate cancer among benign prostatic hyperplasia patients. J Cancer 2014;5:182–91.
[35] Egidi MG, Cochetti G, Guelfi G, et al. Stability assessment of candidate reference genes in urine sediment of prostate cancer patients for mirna applications. Dis Markers 2015;2015:973597.
[36] Mahn R, Heukamp LC, Rogenhofer S, von Ruecker A, Müller SC, Ellinger J. Circulating microRNAs (miRNA) in serum of patients with prostate cancer. Urology 2011;77:1265, e9–16.
[37] Mihelich BL, Maranville JC, Nolley R, Peehl DM, Nonn L. Elevated serum microRNA levels associate with absence of high-grade prostate cancer in a retrospective cohort. PLoS One 2015;10:e0124245.
[38] Nikolić Z, Savić Pavićević D, Vučić N, et al. Assessment of association between genetic variants in microRNA genes hsa-miR-499, hsa-miR-196a2 and hsa-miR-27a and prostate cancer risk in Serbian population. Exp Mol Pathol 2015;99:145–50.
[39] Wach S, A-Janabi O, Weigelt K, et al. The combined serum levels of miR-375 and urokinase plasminogen activator receptor are suggested as diagnostic and prognostic biomarkers in prostate cancer. Int J Cancer 2015;137:1406–16.
[40] Kachakova D, Mitkova A, Popov E, et al. Combinations of serum prostate-specific antigen and plasma expression levels of let-7c, miR-30c, miR-141, and miR-375 as potential better diagnostic biomarkers for prostate cancer. DNA Cell Biol 2015;34:189–200.
[41] Haldrup C, Kosaka N, Ochiya T, et al. Profiling of circulating microRNAs for prostate cancer biomarker discovery. Drug Deliv Transl Res 2014;4:19–30.
[42] Hashemi M, Moradi N, Ziaee SA, et al. Association between single nucleotide polymorphism in miR-499, miR-196a2, miR-146a and miR-149 and prostate cancer risk in a sample of Iranian population. J Adv Res 2016;7:491–8.
[43] Leidinger P, Hart M, Backes C, et al. Differential blood-based diagnosis between benign prostatic hyperplasia and prostate cancer: miRNA as source for biomarkers independent of PSA level, Gleason score, or TNM status. Tumour Biol 2016;37:10177–85.
[44] Pang C, Liu M, Fang W, et al. MiR-139-5p is Increased in the peripheral blood of patients with prostate cancer. Cell Physiol Biochem 2016;39:1111–7.
[45] Sharova E, Grassi A, Marcer A, et al. A circulating miRNA assay as a first-line test for prostate cancer screening. Br J Cancer 2016;114:1362–6.
[46] Wang J, Ye H, Zhang D, et al. MicroRNA-410-5p as a potential serum biomarker for the diagnosis of prostate cancer. Cancer Cell Int 2016;16:12.
[47] Zhang HL, Yang LF, Zhu Y, et al. Serum miRNA-21: elevated levels in patients with metastatic hormone-refractory prostate cancer and potential predictive factor for the efficacy of docetaxel-based chemotherapy. Prostate 2011;71:326–31.
[48] Yun SJ, Jeong P, Kang HW, et al. Urinary microRNAs of prostate cancer: virus-encoded hsv1-mirh18 and hsv2-mir-h9-5p could be valuable diagnostic markers. Int Neurourol J 2015;19:74–84.
[49] Chen ZH, Zhang GL, Li HR, et al. A panel of five circulating microRNAs as potential biomarkers for prostate cancer. Prostate 2012;72:1443–52.
[50] Song C, Chen H, Wang T, Zhang W, Ru G, Lang J. Expression profile analysis of microRNAs in prostate cancer by next-generation sequencing. Prostate 2015;75:500–16.
[51] Choi JK, Yu U, Kim S, Yoo OJ. Combining multiple microarray studies and modeling interstudy variation. Bioinformatics 2003;19(Suppl 1):i84–90.
[52] Walsh CJ, Hu P, Batt J, Santos CC. Microarray meta-analysis and cross-platform normalization: integrative genomics for robust biomarker discovery. Microarrays (Basel) 2015;4:389–406.
[53] Baujat B, Mahé C, Pignon JP, Hill C. A graphical method for exploring heterogeneity in meta-analyses: application to a meta-analysis of 65 trials. Stat Med 2002;21:2641–52.
[54] Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 2000;56:455–63.
[55] Gacci M, Eardley I, Giuliano F, et al. Critical analysis of the relationship between sexual dysfunctions and lower urinary tract symptoms due to benign prostatic hyperplasia. Eur Urol 2011;60:809–25.
[56] Speakman M, Kirby R, Doyle S, Ioannou C. Burden of male lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH)—focus on the UK. BJU Int 2015;115:508–19.
[57] Kirby RS, Kirby M, Fitzpatrick JM. Benign prostatic hyperplasia: counting the cost of its management. BJU Int 2010;105:901–2.
[58] van Exel NJ, Koopmanschap MA, McDonnell J, et al. Medical consumption and costs during a one-year follow-up of patients with LUTS suggestive of BPH in six european countries: report of the TRIUMPH study. Eur Urol 2006;49:92–102.
[59] Cho WC. MicroRNAs: potential biomarkers for cancer diagnosis, prognosis and targets for therapy. Int J Biochem Cell Biol 2010;42:1273–81.
[60] Kim WT, Kim WJ. MicroRNAs in prostate cancer. Prostate Int 2013;1:3–9.
[61] Fang YX, Gao WQ. Roles of microRNAs during prostatic tumorigenesis and tumor progression. Oncogene 2014;33:135–47.
[62] Kristensen H, Haldrup C, Strand S, et al. Hypermethylation of the GABRE ~ miR-452 ~ miR-224 promoter in prostate cancer predicts biochemical recurrence after radical prostatectomy. Clin Cancer Res 2014;20:2169–81.
[63] Carson 3rd C, Rittmaster R. The role of dihydrotestosterone in benign prostatic hyperplasia. Urology 2003;61(4 Suppl 1):2–7.
[64] Gheinani AH, Kiss B, Moltzahn F, et al. Characterization of miRNA-regulated networks, hubs of signaling, and biomarkers in obstruction-induced bladder dysfunction. JCI Insight 2017;2:e89560.
[65] Brower V. RNA interference advances to early-stage clinical trials. J Natl Cancer Inst 2010;102:1459–61.

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