World Journal of Urology (2019) 37:2001–2007
https://doi.org/10.1007/s00345-019-02779-7

TOPIC PAPER

Mutations in CDKN2A and the FGFR3 genes on bladder cancer diagnosis: a systematic review and meta-analysis

Herney Andrés García-Perdomo[1,2] · Juan Pablo Usubillaga-Velasquez[2] · James Alejandro Zapata-Copete[3] · Leonardo Oliveira Reis[4]

Received: 13 November 2018 / Accepted: 20 April 2019 / Published online: 27 April 2019

Abstract

Purpose To determine the association between mutations in CDKN2A and FGFR3 genes and the diagnosis of bladder carcinoma (BCa).

Methods A systematic search strategy was carried out through MEDLINE, EMBASE, LILACS, CENTRAL and unpublished literature. We included RCTs, cohort, case–control and cross-sectional studies that involved patients > 18-year-old assessing the association between CDKN2A and FGFR3 mutated genes and BCa. The primary outcome was bladder cancer defined by histology of the sample. We assessed the risk of bias with QUADAS2 and performed a meta-analysis with Review Manager v5.3.

Results We found 97 records with the search strategies. After duplicates were removed, six studies were included in meta-analysis. Regarding the association between mutated FGFR3 and bladder cancer, we found an OR 31 95% CI (15–64). However, there was no association for CDKN2A and BCa.

Conclusion There is a strong association between FGFR3 mutated gene and the diagnosis of bladder cancer, which has not been observed with CDKN2A. Such a result supports FGFR3 mutated gene as a promissory bladder cancer screening and monitoring biomarker.

Keywords CDKN2A · FGFR3 · Bladder cancer · Genes · Diagnosis

Introduction

Bladder cancer (BCa) is the sixth most frequent type of cancer worldwide, and the fourth in men. BCa had a mortality of 16,870 cases in 2017, around 20% [1]. There are multiple risk factors for BCa such as tobacco smoke, the presence of aromatic amines (dyes, rubber, aluminum workers), chronic infections (schistosomiasis) and/or inflammation of the urinary tract, the presence of arsenic in water and some drugs such as cyclophosphamide.

BCa is considered a public health concern in developed countries, lacking a specific screening and monitoring marker. This is the reason patients only consult when symptoms begin and that is directly related with an advanced disease and bad prognosis, justifying the need to look for development of biomarkers that helps in BCa diagnosis in the near future.

After genomic studies like the Cancer Genome Atlas the understanding of cancer biology has changed with the discovery of large scales of genes expressions that in pathological cases have shown disturbances that can be related with cancer, not as an homogenous disease when the new discoveries demonstrate the heterogeneity in which multiple genetic alteration can lead to tumor advance [2].

Selected polymorphisms of CDKN2A and FGFR3 might have a functional consequence by affecting gene regulation or its protein product and are thus associated with disease

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00345-019-02779-7) contains supplementary material, which is available to authorized users.

✉ Leonardo Oliveira Reis
reisleo.l@gmail.com

[1] Universidad del Valle, Cali, Colombia

[2] Pontificia Universidad Javeriana, Bogotá, Colombia

[3] UROGIV Research Group, Universidad del Valle, Cali, Colombia

[4] UroScience and Pontifical Catholic University of Campinas, PUC-Campinas, Campinas, São Paulo, Brazil

prognosis and diagnosis. Mutations of these genes have been seen as potential biomarkers of common malignancies in blood, urine and tumor tissue. Polymorphic variations in some genes might affect an individual's susceptibility to BCa and the use of this biomarker can improve the management, diagnosis, therapy and prognosis.

The study of genes related to BCa seeks to determine the link to the onset of localized bladder cancer and evaluate the possibility of studying the variations and possible applicability as a biomarker for illness or as an early biomarker for development of the susceptibility to BCa.

The objective of this systematic review was to identify the association between the CDKN2A and the FGFR3 mutated genes and the diagnosis of bladder cancer

Methods

We performed this review according to the recommendations of the Cochrane Collaboration [3] and following the PRISMA Statement [4]. The PROSPERO registration number was CRD42018094610

Eligibility criteria

- Study design: We included clinical trials, cohort, case–control and cross-sectional studies. The studies that were considered were those that included men or women, > 18-year, and looked for the association of mutations in the CDKN2A and the FGFR3 genes, their SNPs and the diagnosis of bladder cancer. Studies from molecular biology, translational and clinical research and those that compared between people with/without BCa were also included.
- Primary outcome: BCa defined by histology of the tumor from transurethral resection.
- Exclusion criteria: observational and descriptive studies; no human subjects.

Information sources

Literature search was conducted in accordance to Cochrane recomendations and medical subject headings (MeSh), Emtree language, Decs and text words related used. We searched MEDLINE (OVID), EMBASE, LILACS and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception to the present. To ensure literature saturation, we scanned references from relevant articles identified through search, conferences, thesis databases, Open Grey, Google Scholar and clinicaltrials.gov, among others. We contacted authors by e-mail in case of missed information. There were no language restrictions.

Additionally, we looked for information in the following specific databases: dbSNP, GeneSNP, Polyphen, Human Genome Database, Ensemble and Nature, among others. The search strategy has been described in Appendix 1.

Data collection

Two researchers reviewed each reference by title and abstract. Then they scanned full texts of relevant studies, applying pre-specified inclusion and exclusion criteria, and extracted the data. Disagreements were resolved by consensus, and when disagreement could not be solved a third reviewer dissolved the conflict.

Two trained reviewers using a standardized form independently extracted the following information from each article: study design, geographic location, authors' names, title, objectives, inclusion and exclusion criteria, number of patients included, losses to follow-up, timing, definitions of outcomes, outcomes and association measures and funding source.

Risk of bias assessment

The assessment of the risk of bias for each study was made using the QUADAS2 tool [5]. We computed graphic representation of potential bias using RevMan 5.3.

Data analysis/synthesis of results

The statistical analysis was performed using Review Manager 5.3 (RevMan® 5.3). For categorical outcomes, the reported information about odds ratio (OR) with 95% confidence intervals was used according to the type of variables and we pooled the information with a random effect meta-analysis according to the heterogeneity expected. The results were reported in forest plots of the estimated effects of the included studies with a 95% confidence interval (95% CI).

Heterogeneity was evaluated using the I^2 test. Values of 25%, 50%, and 75% in the I^2 test correspond to low, medium, and high levels of heterogeneity, respectively. We tried to perform a meta-regression according to the number and the quality of the studies.

Publication bias

Due to the number of studies included, we did not perform publication bias analysis

Sensitivity analysis

We performed sensitivity analysis extracting weighted studies and running the estimated effect to find differences.

Subgroup analysis

We tried to perform subgroup analysis by: NMIBC and MIBC/sample/geographic setting/molecular template. However, we only performed it by sample in the FGFR3 gene.

Results

Study selection

We found 243 records with the search strategies. After duplicates were removed, there were 226 records. Finally, six studies were included in qualitative and quantitative analysis [6–11] (Fig. 1).

Included studies

A total of 1254 patients were included, with a median of 177 patients per study. All six studies evaluated new biomarkers in different samples for early diagnosis of bladder cancer. The studies were performed in the USA, France, Netherlands, Denmark, China and India. Noel [7], Van Kessel [8] and Dahmcke [9] studied the FGFR3 gene and Obaidul Hoque [6], Yang [10] and Hosseini [11] studied the CDKN2A gene (Table 1).

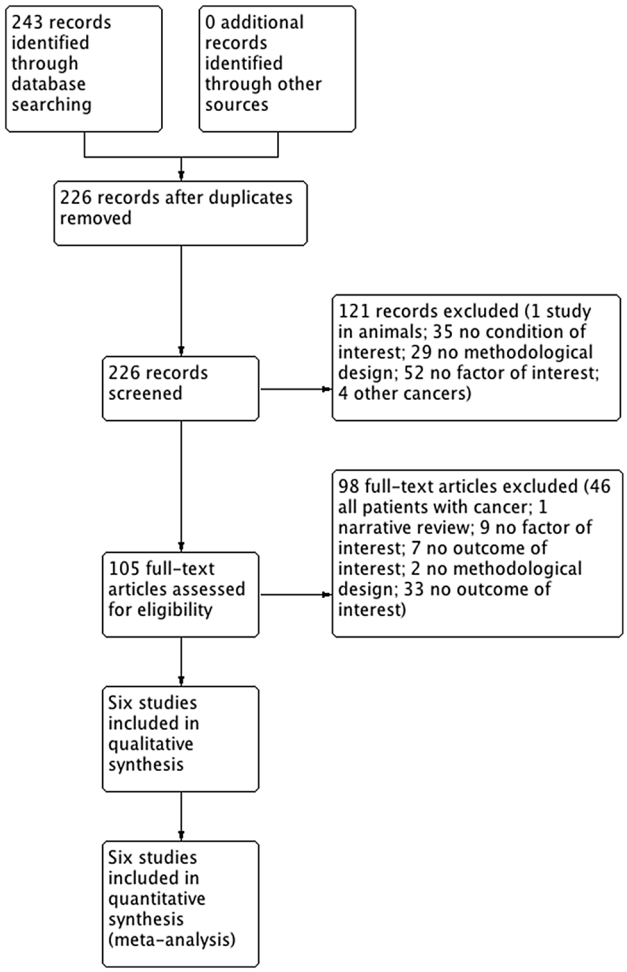

Fig. 1 Flowchart of included studies

Risk of bias assessment

All included studies [6–11] had no information regarding the blinded assessment of the index and reference standard tests; therefore, these two items were graded as unclear risk of bias. On the other side, only two studies (Dahmcke [9] and Van Kessel [8]) were conceived as prospective, leading to low risk of bias; the other studies used a case–control approach. There were no applicability concerns for all studies (Fig. 2a, b).

FGFR3 and bladder cancer

Three studies assessed the association between FGFR3 and bladder cancer (Noel [7]; Van Kessel [8]; Dahmcke [9]). We found an OR 31.22 95% CI (15.11–64.48) I^2 0% (Fig. 3a).

CDKN2A and bladder cancer

Three studies assessed the association between CDKN2A and bladder cancer (Obaidul Hoque [6]; Yang [10]; Hosseini [11]). We found an OR 12 95% CI (0.26–590.79) I^2 87% for tissue samples (Fig. 3b) and an OR 7 95% CI (0.2–258) I^2 84% for blood samples (Fig. 3c).

Sensitivity analysis

We did not find any differences in effect estimate (OR) when we performed sensitivity analysis.

Subgroup analysis

We performed the subgroup analysis for CDKN2A, based on the type of sample (see previously described) (Fig. 3b, c).

Discussion

Summary of the main findings

We found that FGFR3 mutated gene was significantly associated with diagnosing bladder cancer. On the other side, the CDKN2A was not associated with the diagnosis of this kind of tumor with regard to the type of sample analysis.

Table 1 The characteristics of included studies

Author, year	Setting	Age	Gene	Cancer population	No cancer population	Technique	Molecular template	Sample	Cutoff value
Obaidul Hoque, (2006)	USA	58.5 years (28–84)	CDKN2A	160	69	A quantitative fluorogenic real-time polymerase chain reaction (PCR) assay was used to examine primary tumor DNA and urine sediment DNA	DNA	Urine	NA
Noel, (2015)	France	NA	FGFR3	103	5	Mutations were assessed with the SNaPshot system	DNA	Urine	NA
Van Kessel, (2017)	Netherlands	71 (38–110) (Ca); 62 (50–82) (No Ca)	FGFR3	95	99	DNA was isolated using the Qiamp DNA mini-kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. Samples were analyzed for hotspot mutations in FGFR3	DNA	Urine	NA
Dahmcke, (2016)	Denmark	69 (26–91) (Ca); 64 (18–91) (No Ca)	FGFR3	99	376	Cellular DNA was tested for mutations (droplet digital polymerase chain reaction)	DNA	Urine	NA
Yang, (2002)	China	NA	CDKN2A (p16)	67	21	Monoclonal antibody to p16 and cyclin D1	Protein	Tissue	The cutoff limits of cyclin D1 and p16 were set at 10 and 5%, respectively
Hosseini, (2010)	India	57.42 (±12.59) (CA); 56.35 (±10.13) (No Ca)	CDKN2A (p16/p14)	80	80	Methylation-specific PCR (MS-PCR)	DNA	Tissue/blood	NA

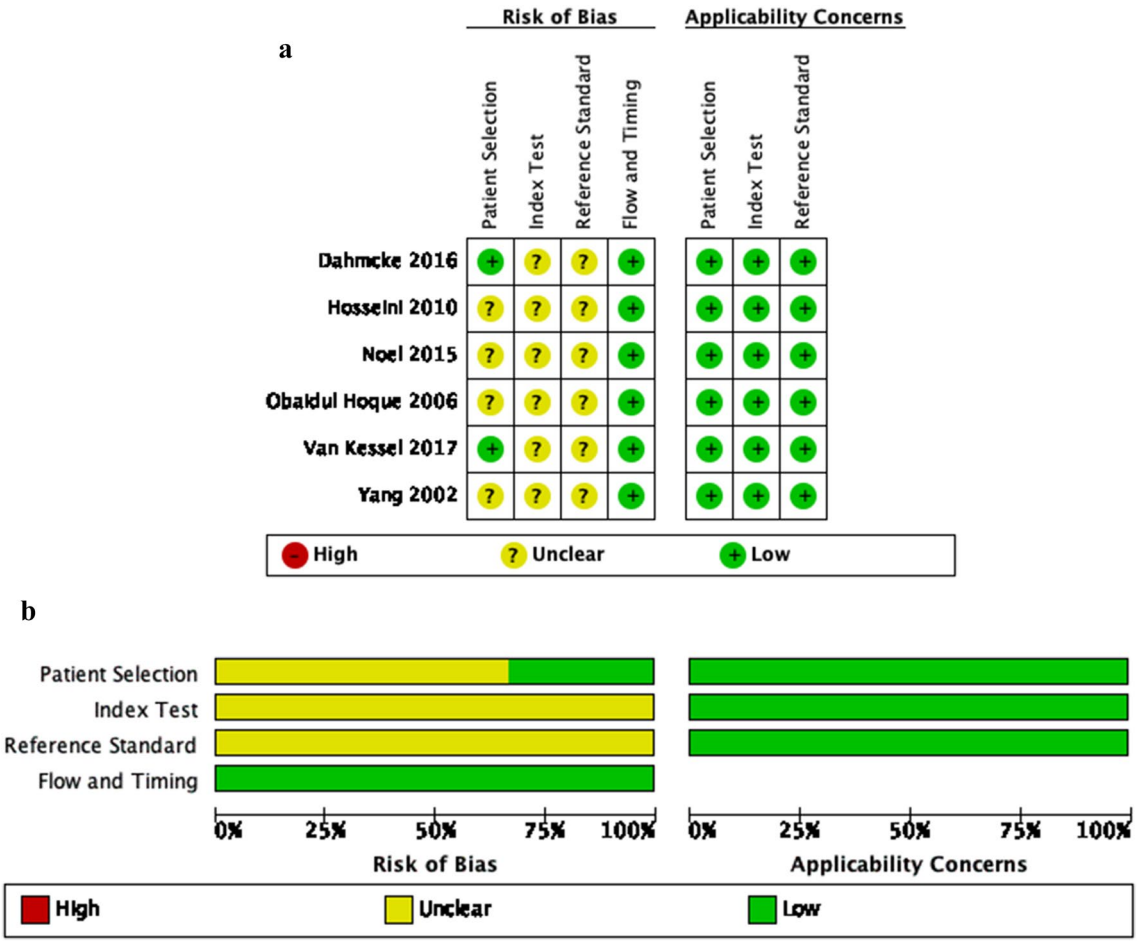

Fig. 2 Risk of bias assessment: **a** within studies, **b** across studies

Contrast with literature

The study of genes related to BCa seeks to determine the link to the onset of localized bladder cancer and evaluates the possibility of studying the variations and possible applicability as a biomarker for illness or as an early biomarker for development of the susceptibility to BCa.

After genomic studies such as the Cancer Genome Atlas, the understanding of cancer biology has changed with the discovery of genes' large scale expressions related to cancer, obviously not as a homogenous disease but in a scenario where multiple genetic alteration can lead to an advanced tumor [12]. Alterations in gene expression patterns, mutations and copy number can determine the grade of malignancy of the tumor, but can also help to find specific therapeutic targets. The genomic and molecular analysis of urothelial tumors has shown a large number of genes that are altered, downregulated or overexpressed that can be directly associated with the development of the illness. Two most representative genes are CDKN2A and FGFR3, which is the reason why we chose to elucidate them in our systematic review.

The CDKN2A is a gene type of cyclin-dependent kinase inhibitor, which is located at chromosome 9 band p21.3 and its mutations have been related with breast cancer, melanoma and squamous cell carcinoma. Recent findings show that from 20 to 60% of bladder carcinomas have CDKN2A gene alteration, and the principal polymorphism is a substitution of alanine to threonine in the second exon causing mutation, deletion or hypermethylation. CDKN2A is an activator of P53, so its alteration may result in a negative control of cell proliferation, in two different ways: first is an inhibition of retinoblastoma protein in G1 cell cycle and the second is binding and inhibiting MDM2 protein that inhibits P53 [13].

The FGFR3 is a fibroblast growth factor receptor 3 encoded in chromosome 4 p 16.3, with ligand affinities and tissue distribution, which interacts with fibroblast growth hormone. Its mutation suggests RAS-MAPK and PI3K-AKT pathways interference. Mutation was seen in 63% of NMIBC

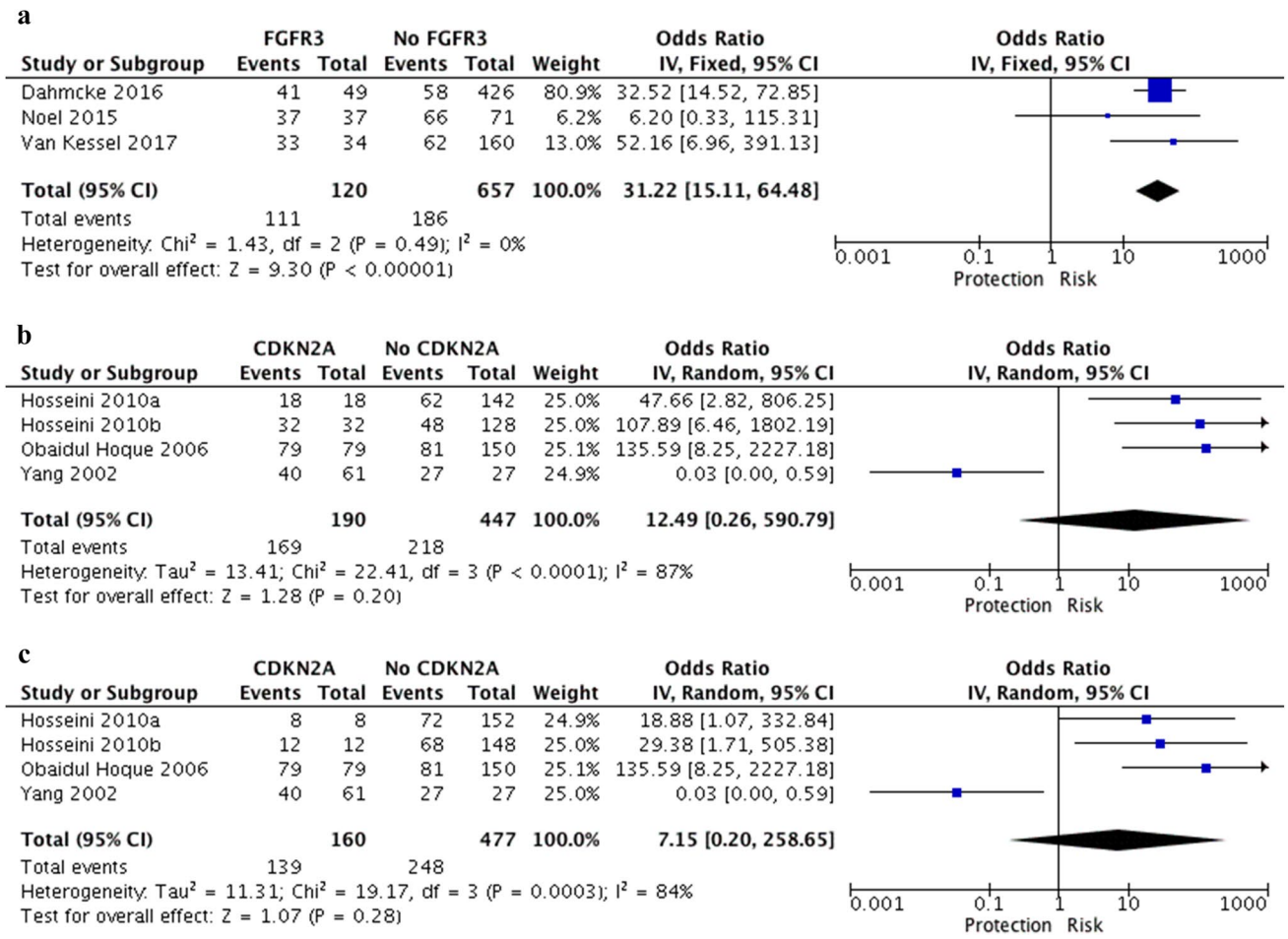

Fig. 3 **a** FGFR3 and bladder cancer. **b** CDKN2A and bladder cancer. Tissue sample. **c** CDKN2A and bladder cancer. Blood sample

and 38% of patients with MIBC, associated with the invasive portion in the muscular layer of the tumor, and also lymph node analysis report was positive for FGFR mutation in 5%. Also it can be analyzed in urinary cells, where its mutations relate to recurrent tumors [12].

While there are three known pillars n BCa genesis, genetic, environmental and immunologic, the most important risk factor recognized so far is limited to the exposure to tobacco, which has more than 400 different carcinogenic compounds. In addition to eventual genetic susceptibility, it might trigger multiple disturbances in cell cycle and chronic inflammation in the urinary tract, increasing by threefold BCa risk. The current study shows that the FGFR3 gene is over ten times stronger to determine BCa risk than exposure to tobacco (OR 31 vs. 3) [14].

Such a result suggests FGFR3 mutated gene to be a promising bladder cancer-specific screening and monitoring biomarker, mainly in the current scenario lacking a strong target to allow an effective screening strategy.

Additionally, despite surgical management with TUR and adjuvant intravesical therapy, about 30% of BCa can result in recurrence of tumor, advancement of the disease, or change in stage, prognosis and mortality. To know which of these patients are more likely to generate recurrence, aggressiveness and how genetic alterations can be diagnosed in a timely manner, and to define patients at risk and even generate straightforward treatment directed at the altered gene remain unanswered questions [15].

Strengths and limitations

This is the first systematic review related to the association of these important genes and bladder cancer, following the international recommendations for systematic reviews and meta-analysis. There was a very sensitive search strategy, enhanced with specific searching in http://Genecards.org, KEGG and UCSC Genome Browser. There was an important effect measure for FGFR3 gene with low heterogeneity, which increases the confidence in this result.

The most important limitation of this review was the high heterogeneity for CDKN2A gene and techniques that could not have been explained.

Clinical and population importance

Based on the findings, mutations in FGFR3 have a direct relationship to diagnose bladder cancer, mainly in patients with risk factors and people with non-invasive bladder cancer. This opens a door for considering the benefit of a screening test to detect patients in early stages and thus preventing them from developing an advanced cancer.

Conclusion

There is an association between FGFR3 mutated gene and the diagnosis of bladder cancer, which has not occurred with the CDKN2A gene. The FGFR3 gene might serve as a putative biomarker allowing future screening strategies.

Acknowledgements We acknowledge the involved institution(s), the patients and those who provided and cared for the study patients.

Author contributions Protocol/project development: HAG and LOR. Data collection or management: HAG, JPU, JAZ and LOR. Data analysis: HAG, JPU, JAZ and LOR. Manuscript writing/editing: HAG, JPU, JAZ and LOR.

Funding Reis LO: CAPES, BEX 14679/13-2 and CNPq Research Productivity, 302622/2015-2, Brazil.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants The PROSPERO registration number was CRD42018094610.

References

1. Spiess PE, Agarwal N, Bangs R, Boorjian SA, Buyyounouski MK, Clark PE et al (2017) Bladder cancer, version 5.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw 15(10):1240–1267
2. Research TCGA (2014) Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 507(7492):315–322
3. Higgins JPT, Green S (eds) (2011) Cochrane handbook for systematic reviews of interventions version 5.1.0 [updated March 2011]. The Cochrane Collaboration. http://handbook.cochrane.org
4. Moher D, Liberati A, Tetzlaff J, Altman D (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ Br Med J 339:b2535
5. Whiting PF, Rutjes AWS, Westwood ME, Mallet S, Deeks JJ, Reitsma JB et al (2011) QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155(4):529–536
6. Hoque MO, Begum S, Topaloglu O, Chatterjee A, Rosenbaum E, Van Criekinge W et al (2006) Quantitation of promoter methylation of multiple genes in urine DNA and bladder cancer detection. J Natl Cancer Inst 98(14):996–1004
7. Noel N, Couteau J, Maillet G, Gobet F, D'aloisio F, Minier C et al (2015) TP53 and FGFR3 gene mutation assessment in urine: pilot study for bladder cancer diagnosis. Anticancer Res 35:4915–4922
8. Van Kessel KEM, Beukers W, Lurkin I, Ziel-van der Made A, van der Keur KA, Boormans JL et al (2017) Validation of a DNA methylation-mutation urine assay to select patients with hematuria for cystoscopy. J Urol 197(3):590–595
9. Dahmcke CM, Steven KE, Larsen LK, Poulsen AL, Abdul-Al A, Dahl C et al (2016) A prospective blinded evaluation of urine-DNA testing for detection of urothelial bladder carcinoma in patients with gross hematuria. Eur Urol 70(6):916–919
10. Yang C, Chu K, Chen H, Chen W (2002) Expression of p16 and cyclin D1 in bladder cancer and correlation in cancer progression. Urol Int 404:190–194
11. Hosseini SA, Sobti RC, Malekzadeh K, Singh SK, Joshi K (2010) Frequency of P16INK4a and P14ARF genes methylation and its impact on bladder cancer cases in north Indian population. Dis Mark 28(6):361–368
12. Felsenstein KM, Theodorescu D (2018) Precision medicine for urothelial bladder cancer: update on tumour genomics and immunotherapy. Nat Rev Urol 15(2):92–111
13. Borkowska E, Jedrzejczyk A, Kruk A, Traczyk M, Pietrusiński M, Marks P et al (2011) The A148T variant of CDKN2A gene in bladder cancer. Hered Cancer Clin Pract 9(Suppl 2):11
14. Rink M, Crivelli JJ, Shariat SF, Chun FK, Messing EM, Soloway MS (2015) Smoking and bladder cancer: a systematic review of risk and outcomes. Eur Urol Focus 1(1):17–27
15. Chang S, Boorjian S, Chou R, Clark P, Daneshmand S, Konety B (2016) Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline. J Urol 196(4):1021–1029