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Systematic or Meta-analysis Studies

The prognostic role of epigenetic dysregulation in bladder cancer: A systematic review

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ABSTRACT

Background: Despite adequate treatment and follow-up, around one fifth of patients with localized bladder cancer will present with disease progression. Adequate prognostic biomarkers are lacking to define patients who are at risk. Mutations in chromatin remodeling genes are more frequently found in bladder cancer than in any other solid tumor. However, the prognostic relevance of epigenetic dysregulation has not been established and may offer an opportunity for biomarker discovery.

Methods: Looking for prognostic epigenetic factors, we performed a comprehensive PubMed search using keywords such as "bladder cancer", "chromatin remodeling", "gene methylation" and "epigenetics". We only included studies reporting on the association of epigenetic markers with prognostic outcomes such as recurrence, progression or survival.

Results: Of 1113 results, 87 studies met the inclusion criteria, which represented a total of 85 epigenetic markers with potential prognostic relevance. No prospective studies were identified. Seventy-three percent (64/87) of the studies involved mixed cohorts of muscle invasive and non-muscle invasive bladder cancer. Promoter methylation of genes with putative prognostic value affected cellular processes such as cell cycle, apoptosis, cell-adhesion or migration, as well as critical pathways such as MAP-kinase or Wnt. Alteration of chromatin regulatory elements suggest a prognostic relevance alterations leading to a predominantly silenced chromatin state.

Conclusions: The prognostic impact of epigenetic alterations in bladder cancer is still unclear. Prospective evaluation of methylation marks and chromatin remodeling gene alterations using consistent methods and criteria is warranted.

Introduction

Bladder cancer is the fifth most common cancer type in both sexes and is more frequent in men, with an estimated 60,490 new cases and 12,240 deaths expected for 2017 in the US alone [1]. Its clinical behavior is usually indolent and, in roughly 70% of cases, presents as a superficial, non-muscle invasive (NMI) tumor that can be cured by transurethral resection (TUR) [2,3]. However, even with adequate treatment and follow-up, progression to muscle-invasive (MI) disease occurs in approximately 21% of patients with high grade disease, which carries a significantly worse prognosis despite aggressive surgical and systemic treatment [3,4]. Thus, it is of utmost interest to identify predictive biomarkers of tumor recurrence and/or progression to help guide clinicians to find optimal treatment strategies.

In bladder cancer, the biological relevance of the hypermethylation of tumor suppressor gene promoters has been widely studied [5,6]. Many investigators have tried to develop gene promoter methylation panels for urine samples as a non-invasive diagnostic method [7]. Also, a large case-control study addressed the potential role of decreased global cytosine methylation in leukocyte DNA and bladder cancer susceptibility [8]. In addition to methylation marks, some studies have assessed the relevance of other genes and proteins with epigenetic regulatory functions, such as chromatin-remodeling genes or non-coding RNAs. Interestingly, chromatin-remodeling gene mutations are highly prevalent in bladder cancer [9–12], which suggests that epigenetic dysregulation is a relevant feature of bladder cancer.

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In this article, our aim was to study the clinical relevance of epigenetic dysregulation in bladder cancer. For that, we performed a systematic search of the PubMed database, focusing on the prognostic value of specific epigenetic markers in bladder cancer patients.

Materials and methods

We performed a systematic literature search of articles published in PubMed up to March 30th 2017. Keywords included "bladder cancer", "transitional cell carcinoma", "chromatin remodeling", "gene methylation" and "epigenetics". We selected those studies that evaluated specific epigenetic marks such as promoter hypermethylation of specific genes and histone tail modifications. We also included studies that evaluated chromatin-remodeling gene alterations and other molecules with a putative epigenetic function, such as transposable elements and non-coding RNAs. Two reviewers (DC and AK) independently screened the abstracts and retrieved full article texts when necessary. Article references were searched to identify additional studies of interest. Studies that evaluated unspecific markers, such as nuclear chromatin shape or global methylation patterns, were excluded. Also excluded were studies that only described the prevalence of the marker(s) of interest and its or their association with other clinical or pathological variables (e.g. tumor stage or grade). Conflicts were resolved by consensus. For each study, basic information including first author name, country and year of publication was extracted and recorded. Additional relevant information included patient number, disease stage, tumor histology, and treatment (Supplementary Table 4). Outcome measures were recurrence and progression rates, recurrence-free and progression-free survival, disease-specific survival, and overall survival. Only markers with relevant prognostic value in at least one study are described in the main text. The detailed search strategy and study exclusion criteria are described in Supplementary Tables 1 and 2. Statistical associations between variables relates to univariate analyses unless stated otherwise in the text, and those corresponding to multivariate analyses are specifically described. The results are organized by pathway or biological function of the markers, which was defined using KEGG, REACTOME and BioSystems annotations (see Tables 1 and 2).

Results

We included a total of 87 retrospective studies, published between 2001 and 2017 (Fig. 1). 26.4% (23/87) of studies involved exclusively non-muscle-invasive bladder cancer (NMIBC) cases and the remaining studies included mixed NMIBC and muscle-invasive bladder cancer (MIBC) patients (Fig. 2). Overall, pathological samples were obtained either by transurethral resection (TUR) or radical cystectomy (RC), and studies including upper-tract urothelial carcinoma (UTUC) cases also included nephroureterectomy samples. Gene methylation was generally measured using methylation-specific polymerase chain reaction (MSP), although some studies also included Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA) gene methylation panels or direct pyrosequencing of bisulfite-treated DNA samples. More recent studies employed also Methylated DNA immunoprecipitation (MeDIP) or chromatin immunoprecipitation (ChIP) (Supplementary Fig. 1).

Some investigators have made a substantial contribution to multiple publications included in this review. In Europe, nine of the thirteen Spanish studies included in this review were led by Dr. Sánchez-Carbayo from CNIO (Spanish National Cancer Research Centre) in Madrid [13–21], of which four were focused on NMIBC [13–16]. Secondly, four out of five British studies were led by Dr. James Catto, from the University of Sheffield (UoS) [22–25]. Thirdly, six of the nine German studies have been published by Dr. Kurt Miller's team at Charité-Universitätsmedizin Berlin [26–31]. Finally, all of the Portuguese studies were conducted by Dr. Carmen Jerónimo's group in IPO (Portuguese Oncology Institute) [32–36]. Regarding Asian studies, significant contributions have been made by Dr. Ying-Li Lin [37–43] at Jiangsu University and Dr. Liqun Zhou (especially in UTUC studies) [44–46] at Peking University in China, as well as by Dr. Wun-Jae Kim at Chungbuk National University in South Korea [47–54]. References to the race of the patients are made throughout the text to describe the results in a more organized manner and put them in context.

Gene promoter methylation

Cell-cycle genes

The Cyclin-Dependent Kinase Inhibitor 2A (*CDKN2A*) gene encodes two structurally unrelated protein products, p16 and p14 [55]. Methylation of *p14* was associated with poor overall survival (OS) in Japanese patients ($p = .029$) [56]. A study by Dominguez et al. involving a Spanish mixed population cohort found higher recurrence rates (RR) for those patients whose tumors harbored either *p16* promoter hypermethylation alone ($p = .001$) or concomitantly with *p14* promoter methylation ($p = .01$) [57]. Furthermore, UTUC patients with pTa disease and *p16* methylation had an increased risk of progression ($p = .03$) in one of the UoS studies [22]. However, many other investigations failed to find a significant prognostic value for *p16*/*p14* promoter methylation in bladder cancer [13,14,25,41,58–65].

RB1 (Retinoblastoma 1) promoter methylation had different effects in Spanish NMIBC patients, being associated with a higher risk of recurrence in one study (where *CHFR* – Checkpoint With Forkhead And Ring Finger Domains – methylation was also an adverse factor for recurrence) [13], but protective for progression free survival (PFS) in another ($p < .05$ for both) [14]. In another CNIO NMIBC cohort, Polyamine-modulated factor 1 (*PMF1*) methylation was associated with better recurrence free survival (RFS) and PFS ($p = .04$ and $p = .02$, respectively) [15].

Cell-adhesion

E-cadherin (*CDH1*) methylation has been reported as predictive of poor PFS (Multivariate analysis [MA]: $p = .02$) [25] and overall survival (OS) (MA: $p = .02$) [66]. However, *CDH1* methylation was associated with lower progression rates ($p = .49$) in the first study published by Dr. Catto [22]. Moreover, others have failed to report a prognostic value for this marker [44–46,60,67].

The studies by Dr. Lin at JU have reported that methylation of *CDH13* (Cadherin 13) is associated with increased recurrence risk ($p = .0043$), shorter time to progression ($p = .006$), lower RFS ($p < .0001$) [43] and with an increased risk of death ($p = .0071$) [41]. In another of his studies, *CDH11* methylation correlated with poor OS ($p = .0004$) [40]. However, *CDH13* methylation had no prognostic value in other cohorts [13,66]. Dr. Lin's group also reported that protocadherin genes *PCDH17* and *PCDH10* methylation are strong predictors of poor OS ($p < .05$ for both) [37,42]. Furthermore, he reported that methylation of another protocadherin gene, *PCDH8*, predicted poor RFS in both NMIBC and mixed population studies [38,39].

Dr. Sánchez-Carbayo's group reported lower progression rates for patients with *THBS*1 (Thrombospondin 1) methylation alone or combined with that of other genes such as *RB1, TP73* (Tumor Protein P73) or *MSH6* (MutS Homolog 6) ($p < .05$ for all statistical