

OPINION ARTICLE

DNA Methylation and Bladder Cancer: Where Genotype does not Predict Phenotype

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Abstract: Nearly three decades ago, the association between Bladder cancer (BC) and DNA methylation has initially been reported. Indeed, in the recent years, the mechanism connecting these two has gained deeper insights. Still, the mediocre performance of DNA methylation markers in the clinics raises the major concern. Strikingly, whether it is the inter-individual methylation variations or the paucity of knowledge about methylation fingerprints lying within histologically distinct subtypes of BC requires critical discussion. In the future, besides identifying the initial causative factors, it will be important to illustrate the cascade of events that determines the fraction of the genome to convey altered methylation patterns specific towards each cancer type.

Keywords: Bladder cancer, DNA methylation, epigenetic, cancer biomarkers, genotype, phenotype.

1. INTRODUCTION

Bladder cancer (BC) is the 9th most common malignant tumor worldwide. BC is more frequent in males, but females have a higher mortality rate [1, 2]. Over the years, the sustained attention of cancer epigenetics has drawn the major interest towards BC, specifically in context to DNA methylation. This can be evident from the pioneering work by Del Senno who identified a correlation of BC and methylation of the *c-myc* proto-oncogene [3]. A decade later, DNA methylation inhibitors have been used to inhibit DNA methylation in bladder tumor cell lines by Bender and colleagues [4]. The advancement of techniques over the years identified global methylation changes (immunohistochemistry), single loci/promoter methylation (methylation specific PCR, pyrosequencing) and established a clear link between DNA methylation and BC [5-7].

Furthermore, in the post-genomic era, massive methylation sequencing and bioinformatics analysis have enhanced the genomic insights for this particular disease. Still, no good biomarker is available that could predict the progression of this disease. By examining several genes simultaneously, we have already acquired lots of information about their hypo/hypermethylation status in BC (Fig. 1). However, many of these candidate genes are known to be involved in other cancer types as well *e.g.*, *CDKN2A* gene, apart from BC its alterations (promoter hypermethylation) have been reported in several other cancers [8]. In BC, administration of

SGI-110 (Guadecitabine, a DNA methyltransferase inhibitor) and 5-aza-2'-deoxycytidine in murine xenograft models exclusively reduced the methylation status of the *CDKN2A* promoter but did not reduce the tumor growth [9]. After years of data accumulation, to the best of our knowledge, only a single clinical study (NCT00978250) has been implicated which currently is still under review (clinicaltrials.gov). This, in turns, raises the concerns about the mediocre performance of DNA methylation in clinics. Until recently, where the study shows that T_{RM} cells residing in the tumor tissue of urinary bladder cancer (UBC) patients have low DNA methylation in the *PRFI* locus, hence, proposed these T_{RM} cells as new targets for cancer immunotherapy [7].

The major discrepancies one could think of might be: inter-individual methylation variations, molecular heterogeneity within bladder tissues, treatment variability, biological age heterogeneity, genes susceptible to gain methylation, ethnic background, or the material/cell lines variability. Hynds and colleagues recently, discussed the scale of heterogeneity between strains of cancer cell lines and the accumulation of heavy mutational load over the last decades [10]. Similarly, tumor specific variable methylation levels within neighboring CpGs embedded within long interspersed nucleotide elements (*LINE-1*) for cancers has also been recently discussed [11]. One could notice that it could also be the diverse histological characterization of BC subtypes, such as urothelial cell carcinoma (UCC), squamous cell carcinoma, papillary carcinoma and adenocarcinoma are named few (Fig. 1). Additionally, rare diseases of the bladder (*e.g.* classical bladder exstrophy) where long term complications can increase the risk of bladder cancer cannot be excluded [12]. Studies have shown that noninvasive DNA methylation

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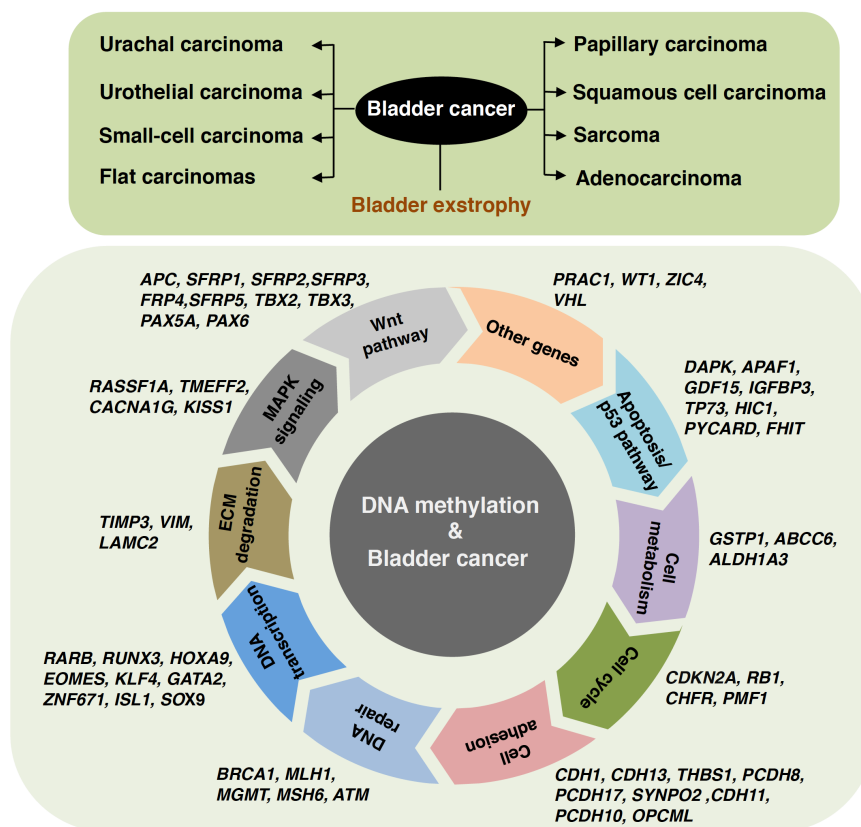


Fig. (1). Histologically distinct bladder cancer subtypes and rare exstrophy of the bladder are shown (upper section). The known genes with altered DNA methylation levels in bladder cancer are classified (lower section, adapted from Casadevall *et al.* [17]). (A higher resolution / colour version of this figure is available in the electronic copy of the article).

markers in biological fluids (blood, urine) can act as a predictive marker up to certain extent [13, 14]. However, considerable methylation differences in these pre-diagnostic tests already indicates that molecular damage has already been done, hence left no choice except treatment improvement for patients to prolong their survival.

Despite a large number of studies that have addressed the local/global DNA methylation changes in multiple human cancers and other diseases [11, 15, 16], the key link showing the switch towards the dynamics of DNA hypo-/hypermethylation is still missing. Perhaps, defining the distinct roles of hypo- and hypermethylation within the same individual might help to undermine their potential consequences. In this scenario, familial cases can reveal more information about the methylation plasticity as compared to the random sporadic cases. Furthermore, genetic subtypes of bladder cancer that might differ from each other in response to various treatments should be openly discussed. To this end, whether the healthy control individuals (used to compare with patients) have their tendency towards getting similar diseases or not should be excluded. It is also noteworthy to mention that the computational tools using publically available cancer-related datasets should be focused on clinically well-defined tissue samples only, instead of analyzing a heterogeneous mixture of tissue subtypes. On the broader range, apart from tumor suppressor genes, why only the fraction of genes displays altered methylation patterns specific to each cancer type, holds the key for molecular dissection of the cancer genome.

CONCLUSION

At the molecular level, it is primarily the histologically distinct subtypes of the bladder that can help to refine our understanding of the functional link between genetic and epigenetic heterogeneity.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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