Chapter

Gender Disparities in Bladder Cancer

Yingsheng Zhang, Dan Theodorescu and Xue Li

Abstract

Biological sex is an independent risk factor of cancer. Men are three to five times more likely than women to develop bladder cancer even when known risk factors are taken into consideration. Development of sex in mammals is often viewed as a two-step process. The first step is sex determination, of which the XX and XY sex chromosome complements trigger gonad differentiation to form the ovary and testis, respectively. After that, sex hormones secreted by gonads initiate sexually dimorphic differentiation of nongonadal tissues. However, this model has been challenged by recent findings revealing an independent contribution of sex chromosomes to sexual dimorphism. In this chapter, we discuss how the sex chromosomes and sex hormones together cause gender disparities in bladder cancer. We propose a concept of epigenetic sex – epigenetic differences between males and females – and suggest that the sex epigenome is a previously unknown biasing factor contributing to gender disparities in bladder cancer.

Keywords: Bladder Cancer, Gender Disparities, Sex Hormones, Sex Chromosomes, Sex Epigenome, KDM6A, PRC2, COMPASS

1. Introduction

Bladder cancer (BC) originates primarily from the urothelium – the inner lining of bladder lumen. Men are disproportionally affected by the disease. Males are 3–5 times more likely than females to BC [1]. This difference persists even after adjusting other known risk factors [2–5], suggesting that male sex is an independent risk factor of BC.

Typical males have one copy each of the X and Y chromosomes (XY) while females have two copies of the X chromosome (XX). For XY individuals, sex-determining region Y (SRY) gene on the Y chromosome triggers gonadal differentiation to form testes, which secrete androgens and promote male primary and secondary sex characteristics. Females with XX chromosome complement have ovarian development and estrogen secretion leading to female primary and secondary sex characteristics [6].

Strong evidence exists that androgens acting through androgen receptor, promote bladder tumorigenesis [7]. Female gonadal hormones acting through estrogen receptors also influence BC risk albeit playing a minor role when compared to androgens [8]. While sex hormones clearly play important roles in gender disparities in BC, potential role of the sex chromosomes is not nearly as apparent. Because the sex chromosomes (*i.e.,* XX vs. XY) are coupled with the gonadal hormones (*e.g.*, estrogens vs. androgens), it is exceedingly difficult to ascertain independent effects of the sex chromosomes or the gonadal hormones [6]. Creative tools are needed

to overcome the challenges of studying sex differences in physiology and disease [9]. Here, we review how the sex hormones and chromosomes function together to cause gender disparities in BC and, furthermore, propose a novel concept of epigenome sex.

2. The impact of biological sex on bladder cancer development

2.1 The role of androgen

A retrospective study revealed that male patients who received α-reductase inhibitors before the diagnosis of BC had better survival and was positively correlated with duration of administration [10]. Similarly, prostate cancer patients who received androgen deprivation therapy (ADT) had a lower incidence of BC compared with patients that did not [11]. Paradoxically, a majority of expression analyses of androgen receptor (AR) in BC patients showed a negative correlation between AR expression and the aggressiveness of BC [12–17]. Offering a possible explanation underlying this observation is our finding that reduced AR expression may lead to upregulation of cancer stem cell related genes such as CD44 [18]. Clearly the role of androgens and AR in BC is complex and likely in molecular and cellular context-dependent manner.

Animal models of BC support the role of androgens/AR in modulating bladder tumorigenesis. Male mice were much more vulnerable than female mice to BC induced by N-butyl-N-(4- hydroxybutyl) nitrosamine (BBN), a bladder-specific carcinogen [19]. This sex difference was blunted by castration. Similarly, Okajima and colleagues have observed male-biased responses to BBN-induced BC in rats. Moreover, administration of Diethylstilbestrol (DES), a nonsteroidal estrogen, suppressed bladder carcinogenesis in male rats. In contrast, testosterone supplementation increased the incidence of BC in female rats [20]. Miyamoto *et al.* have also found that AR knockout (KO) mice, both males and females, were completely protected from BBN-induced BC [21]. Intriguingly, 25% AR KO male mice supplemented with dihydrotestosterone (DHT) had BC after BBN treatment, and 50% of castrated male mice still developed into BC [21]. These data suggest that androgens have AR-independent function in promoting BBN-induced BC, and AR may also function in an androgen-independent manner. In addition, they showed that androgen deprivation or androgen/AR disruption blunted growth of AR-positive human BC cells both *in vitro* and *in vivo* [21]. The genetic evidence of oncogenic role of androgen/AR signaling in bladder carcinogenesis was further confirmed in the subsequent studies. Hsu and colleagues revealed that urothelium-specific conditional KO of AR reduced the risk of BBN-induced BC in male mice. They further showed that AR expression was inversely associated with the level of p53-PCNA-DNA damage pathway, implicating a possible downstream event of AR oncogenic signaling [22]. Johnson *et al.* showed that conditional overexpression of human AR in mouse urothelium was sufficient to promote BBN-induced BC in both sexes and the phenotype in males could be alleviated by castration [23]. Surprisingly, the study also suggested that AR did not function through known molecular pathways which have been previously implicated, including *p53* [22], *Wnt/β-catenin* [24, 25], and *CD24* [26–28]. Therefore, much more work is needed to define the precise role of AR in bladder tumorigenesis.

2.2 The role of estrogens and their receptors

Postmenopausal women had a higher risk of BC and early menopause enhanced this risk, suggesting that female sex hormones protect women from BC

development [29–31]. Classical estrogen receptors (ERs) include estrogen receptor α (ERα) and estrogen receptor β (ERβ) [32]. Clinically, inconsistent results existed between the expression of ERα and ERβ and the grades and stages of BC patients although more reports supported that expression of ERα favors a better while ERβ is associated with a worse prognosis [14, 32–38]. Experimentally, both whole body ERα KO or urothelium-specific ERα KO increased the incidence of BBN-induced BC in female mice. Disruption of ERα decreased the expression of Inositol polyphosphate-4-phosphatase, type II (INPP4B), resulting in a higher activation of AKT [39]. On the contrary, whole body deletion of ERβ impeded BBN-induced BC in female mice [40]. Moreover, knockdown (KD) of ERβ suppressed transformation of normal bladder cells and growth of BC cells partly through reducing expression of mini-chromosome maintenance complex component 5 (MCM5) because reintroduction of MCM5 into BC cells blunted ERβ KD phenotype [40]. Interestingly, tamoxifen treatment conferred a chemoprevention in female mice against BBN-induced BC [41]. Since tamoxifen is a selective estrogen-receptor modulator with mixed estrogenic and antiestrogenic activity depending on targeted tissues, it would be interesting to see which ERs, ERα or ERβ, is activated or inhibited and whether any of these receptors plays a more dominant role in the BBN-induced bladder carcinogenesis.

2.3 The role of sex chromosomes in driving gender disparities

A potential role of the sex chromosomes in gender disparities in BC was implicated initially by cancer epidemiological findings of Turner and Klinefelter patients. Turner syndrome is a genetic disorder of female X0 patients who lost one copy of the X chromosome. Conversely, Klinefelter syndrome has two or more copies of the X chromosome among the affected male patients. Turner patients displayed an increased risk of BC [42]; and Klinefelter patients had an overall reduced rate of solid tumors [43]. These observations suggest that an extra copy of X chromosome is tightly associated with low BC risk in both sexes. However, because the sex chromosomes are tightly coupled with their respective sex/gonadal hormones, the confounding effect of sex chromosomes cannot be excluded. As a result, the sex hormone–independent roles of the sex chromosomes have largely been overlooked.

To overcome the aforementioned challenges of studying independent roles of the sex-biasing factors, De Vries *et al.* developed "four-core genotype (FCG)" mouse model to uncouple the link between the sex chromosomes and their corresponding gonadal types [44]. In this model, *Sry* gene is "transferred" from the Y chromosome to an autosome. As a result, the FCG mouse model has four instead of two sex types: 1. XY male with testes (XYM); 2. XX male with testes (XXM); 3. XX female with ovaries (XXF); and 4. XY female with ovaries (XYF). Independent effects of the sex hormones can be evaluated by comparing XXF and XXM mice or XYM and XXM mice without the confounding issue of the sex/gonadal hormones [45]. Similarly, in the comparisons of XXF vs. XYF or XYM vs. XFM, one can evaluate independent effects of the sex chromosomes.

By taking advantage of the FCG mice, we showed that, independent of the sex hormones, the sex chromosomes had a sex-biasing effect on BC development [46]. We further showed that regardless of gonadal sex XY mice had 2.55 times of higher chance of developing BBN-induced BC than XX mice, demonstrating an independent role of the sex chromosomes. This study has also confirmed the sex-biasing role of androgens and further revealed that having testes was 4.71 times more likely than having ovaries to develop BC. More strikingly, wild type male mice with the XY chromosome complement and testes were 12.39 times more likely than wild type female mice with the XX chromosome complement and ovaries. This is unexpected

because it is close to the product of 2.55 and 4.71 instead of the sum. Such finding suggests that both the sex chromosomes and the sex hormones have independent and dependent sex-biasing effects on BC. Moreover, the sex chromosomes interact with the sex hormones to amplify the difference (**Figure 1**). The underlying mechanism of interaction between these sex-biasing factors is yet to be defined.

Because the Y chromosome is frequently lost in BC cells and its loss has been associated with a higher cancer risk [47–49], it is less likely that the Y chromosome explains the male dominance in BC. A more reasonable possibility is that copy number difference of the X chromosome may render females better protected than males. To understand the potential tumor suppressing role of the X chromosome, we have examined the X chromosome-linked genes that escape X chromosome inactivation (XCI) (**Figure 1a**) [50], hence, genes that are expressed in higher levels in XX than in XY urothelial cells. By comparing gene expression levels in the bladder urothelium of FCG mice, we have identified Lysine Demethylase 6A (KDM6A)

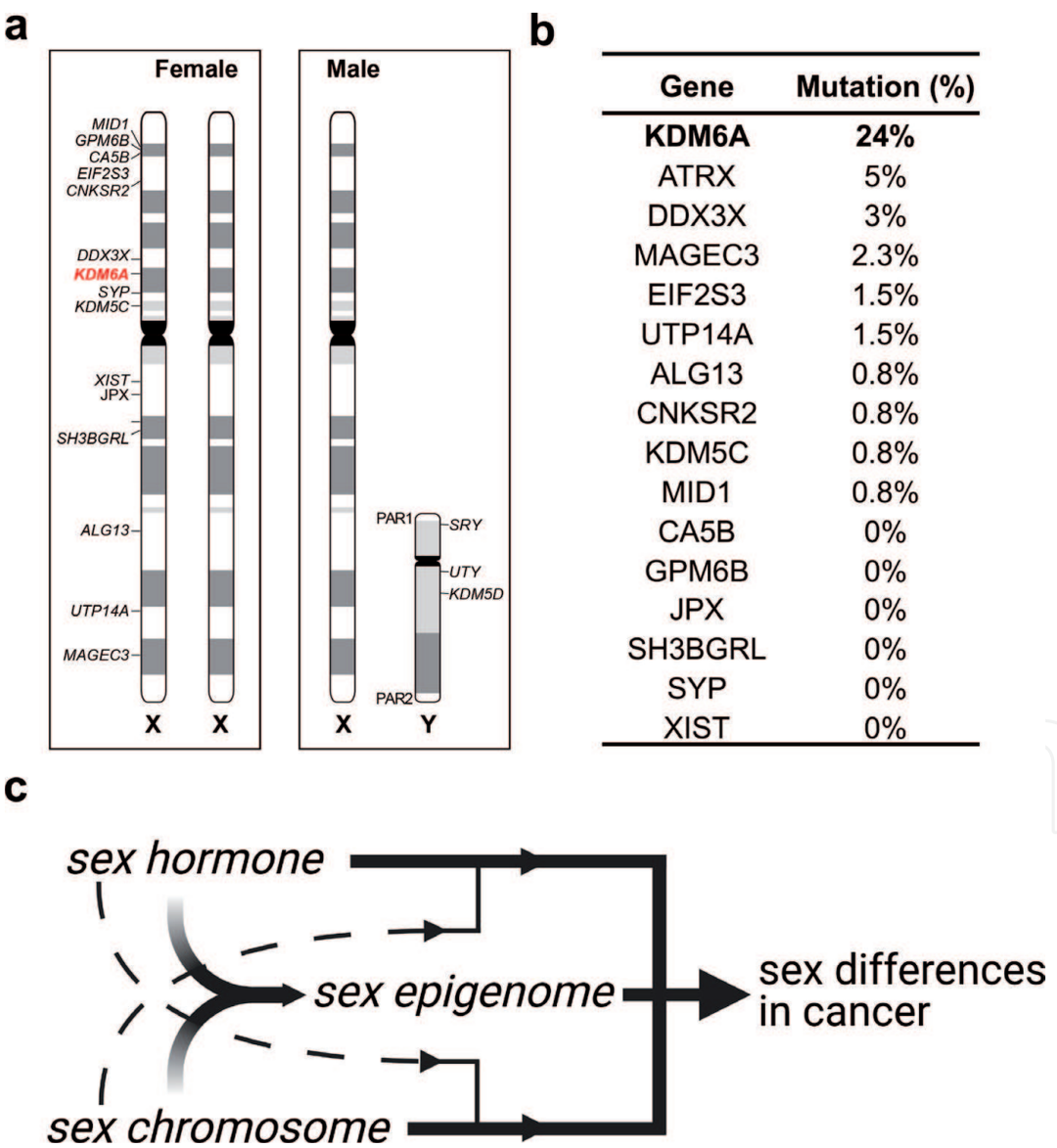

Figure 1.
Major contributing factors of sex differences in bladder cancer. a) Schematic diagram of human sex chromosome complement of XX females and XY males. Genes that are reportedly escaped X-chromosome inactivation (XCI) in both mouse and human are indicated. PAR, Pseudo Autosomal Regions. b) Somatic DNA mutation rate of the candidate XCI escapees in bladder cancer. c) A proposed model that interactions among the sex chromosomes, sex hormones, and sex epigenome amplify male and female differences in bladder cancer.

as a top candidate of X-linked tumor suppressors [46]. *KDM6A* encodes a histone demethylase to remove methyl group from methylated histone H3 at lysine K27 to allow gene transcription. Somatic loss-of-function mutations of human *KDM6A* are tightly associated with BC, suggesting a tumor suppressive function in humans [51–54]. Interestingly, *KDM6A* mutations have been shown to be more common in female patients with non-muscle invasive BC (NMIBC) [54]. We showed that after conditional *Kdm6a* KO in the urothelium of female mice, susceptibility to BBN-induced BC was significantly increased. Similarly, down-regulation or mutation of *KDM6A* was tightly linked to advanced disease stages and poor outcomes among female but not male BC patients [46]. Urothelium-specific *Kdm6a* KO did not exhibit apparent phenotype in male mice. A lack of phenotype in males is potentially due to the compensatory effect of a paralog gene *UTY* on the Y chromosome, albeit that UTY lacks any detectable demethylase activity *in vivo* [55–58]. In addition to *KDM6A,* other X-linked genes have also been suggested to contribute to sex differences in cancer [59]. While these candidate genes were expressed in higher levels in females than in males, their expression was not associated with disease outcomes of BC (**Figure 1b**) [46]. Collectively, these findings suggest that *KDM6A* is a prototypical sex-biasing tumor suppressor in BC.

2.4 The sex epigenome

Through the intrinsic histone demethylase activity, KDM6A regulates downstream gene transcription by antagonizing Polycomb Repressive Complex 2 (PRC2)-dependent epigenetic gene silencing program [60–63]. Specifically, KDM6A catalyzes removal of the methyl groups from histone H3 lysine 27 trimethylation (H3K27me3), making H3K27 available for acetylation (H3K27ac). H3K27me3 and H3K27ac are closely associated with transcription repression and activation, respectively. In a demethylase-independent manner, KDM6A functions in the COMPASS-like protein complex that harbors MLL3/KMT2C and MLL4/KMT2D lysine methyltransferases [64]. KMT2C and KMT2D catalyze formation of H3K4 monomethylation (H3K4me1), which is tightly linked to active transcription enhancers [65–67]. COMPASS and PRC2 display an antagonistic relationship in regulating downstream gene expression [68, 69]. Therefore, *KDM6A* plays a central role in shaping the epigenetic landscape by modulating the PRC2-dependent gene silencing and the COMPASS-dependent gene activation. Nearly 74% of NMIBC and 23% MIBC patients have mutations in *KDM6A* [51, 54]. About 18% and 28% of human BC patients have somatic mutations in *KMT2C* and *KMT2D*, respectively [51]. The sex-biased expression of *KDM6A* suggests that there is an epigenetic difference in bladder urothelium between sexes, hence the sex epigenome. It is conceivable that *KDM6A* plays a critical role in creating an epigenetic barrier to prevent malignant transformation. Females with more *KDM6A* may have a tougher barrier than males to overcome, thereby are less likely to develop BC.

Whole-genome transcriptome analysis of *Kdm6a* knockout urothelium indicates that the p53 tumor suppressor pathway is among the top affected pathways [46]. The canonical p53 downstream gene targets, *Cdkn1a* and *Perp*, which induce cell cycle arrest and apoptosis, respectively, are down-regulated significantly in *KDM6A* knockouts. UM-UC-13, a human BC cell line, lacks functional *KDM6A* due to genomic mutation [70]. Expression of wild type *KDM6A* induces both *CDKN1A* and *PERP* in UM-UC-13 cells while expression of the catalytically-dead form of *KDM6A* only induces *PERP* but not *CDKN1A*, suggesting that *KDM6A* regulates *PERP* gene expression in a demethylase-independent manner. While persistent expression of the enzymatically-dead form of KDM6A is required to suppress UM-UC-13 cell proliferation, a transient expression of *KDM6A* is sufficient to

achieve the same result, implying that the demethylase activity of KDM6A results in a lasting protective activity or an epigenetic memory of tumor suppression [46]. In supporting this notion, *Ler et al.* revealed that *KDM6A* mutant BC cells are more vulnerable to the pharmacological inhibition of PRC2 [52]. While the precise mechanism of *KDM6A*-dependent tumor suppression remains to be fully elucidated, we suspect that systematic profiling of the sex epigenome would shed new light on the gender disparities in BC (**Figure 1c**).

3. Conclusion

A new concept of sex epigenome begins to emerge. In addition to gonadal hormones, the copy number difference of the X chromosome between males and females contributes to sex differences in BC - an extra copy of the X chromosome confers a better protection of females. Moreover, there is a cooperative interaction between the sex hormones and chromosomes during BC development. The tumor suppressing effect of the X chromosome is largely mediated by *KDM6A*-dependent epigenetic program, or the sex epigenome. The sex chromosome, sex chromosome, and sex epigenome collectively contribute to sex differences in BC.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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