Sex discrepancy characterization revealed by somatic DNA alterations in muscle-invasive bladder carcinoma

Tian-Zhi Wu¹, Cheng-Yong Lei², Jin-Ming Li¹, Yan-Fang Guo¹

¹Institute of Bioinformatics, School of Basic Medical Science, Southern Medical University, Guangzhou, Guangdong 510515, China; ²Department of Urology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong 510515, China.

To the editor: Bladder cancer exhibits significant sex differences in incidence, diagnosis, management, and survival.¹ The incidence of bladder cancer in males is approximately three-fold higher than that in females. Although women are generally less likely to develop bladder cancer than men, once females acquire this disease, the prognosis is less favorable. However, the molecular basis of these sex disparities in bladder cancer remains poorly understood. Studies suggest that bladder cancer has more somatic DNA alterations than almost any other cancer type, but the specific differences between males and females remain unknown.² To determine the genomic mutations of sex discrepancies in bladder cancer, we investigated sex-specific somatic DNA alterations by analyzing high-throughput data obtained from The Cancer Genome Atlas (TCGA), including 304 Caucasian samples of muscle-invasive bladder carcinoma (224 males and 80 females).

Overall, males displayed a significantly higher mutation rate than females. First, sex differences in mutation rate were detected according to five categories of mutation types (Tp+C->T/G, Tp+C->A, [A/C/G]p+C->mut, A->mut, and null + indel). The total mean mutation rates for the male and female samples were 4.10 and 3.59 per megabase, respectively, demonstrating a significant difference (Fisher exact P < 2.20 × 10⁻¹⁶). Four (Tp+C->T/G, Tp+C->A, [A/C/G]p+C->mut, A->mut) of the five mutation types showed significantly higher mutation rates in males than in females, with a Fisher exact test P value below 4.65 × 10⁻⁹. Notably, the most significant mutation rate difference between the two sexes was demonstrated by mutation type Tp+C->T/G (P < 2.20 × 10⁻¹⁶), which had high mutation rates for both sexes (relative rate: 4.68) and females (relative rate: 3.99). Second, sex differences in mutation rate were also detected according to individual somatic mutation rates. The results also showed a difference between the two sexes (Wilcoxon rank-sum test P = 0.01, with a mean and median number of somatic mutation variants of 281.4 and 205.5, respectively, for males and 229.2 and 157.5, respectively, for females. Third, the cumulative gene mutation rates were higher in men than those in women. For male samples, a total of 63,038 mutations (60,257 point mutations and 2779 insertions or deletions) were identified for 17,286 genes, of which 264 genes had cumulative mutation rates greater than 5% (approximately 1.40%). Of the ten genes with the highest mutation rates in the male and female samples, seven genes (TTN, TP53, KDM6A, ARID1A, MUC16, KMT2E, and PIK3CA) were found for both sexes, and only three genes each were unique to the male (SYNE1, KMT2C, and GRG1B) and female (FAT1, MUC17, and HMCN1) samples. Among the above genes, TP53, KDM6A, ARID1A, and PIK3CA have been implicated as genes significantly related to bladder cancer in previous studies. We further screened the genes with statistically significant differences in gene mutation rates (greater than 5%) between the male and female samples using both the Fisher exact test and propensity scores. After a strict Bonferroni correction for multiple comparisons, nine genes (SYNE1, MUC5B, FRY, HERC2, PARD3, ATR, DMXL1, SUPT16H, and CDH23) were identified as having significantly different gene mutation rates between the two sexes by both methods, with smaller P values for propensity score testing after accounting for possible confounding factors, such as age, cancer stage, and smoking status. Interestingly, all genes except for F8 demonstrated higher mutation rates in men than in women. The mutation rate of the SYNE1 gene, which was among the top ten genes with the highest mutation rate in males, was significantly different between males and females.

Sex-specific mutated genes with recurrent somatic mutations were detected. Twenty-three genes in the male sample and eleven genes in the female sample showed significant numbers (q ≤ 0.05) of recurrent somatic mutations after analysis by MutSigCV. Eight genes (CDKN2A, KDM6A,
TBC1D12, TP53, PIK3CA, RB1, ELF3, and ZFP36L1) were significantly mutated in both the male and female samples. Sex-specific genes with recurrent mutations were also detected, including eighteen genes (ARID1A, CDKN1A, STAG2, TSC1, RHOB, TXNIP, RBM10, PARD3, HLA-A, EP300, C3orf70, NUDT11, ASXL2, ZFP36L2, and PTEN) that were observed only in the male sample and three genes (HRAS, NFE2L2, and FBXW7) that were observed only in the female sample. Four genes (TXNIP, NUDT11, and ZFP36L2 in the male sample and

Figure 1: Graphical representation of significant amplification and deletion events in males (blue) and females (red). GISTIC 2.0 was used to identify statistically significant focally amplified (A) and deleted (B) regions, which are plotted on the genome scale with chromosome numbers. The scale in red and blue is the G score from GISTIC.
FBXW7 in the female sample) were detected as significantly mutated in sex-specific samples or in the overall sample. Eleven genes (TBC1D12, HLA-A, ASXL2, RBM10, PTEN, C3orf70, PSIP1, MARK2, PARD3, FOXE1, and KRTPA4-7) have not been reported in previous TCGA bladder cancer studies.

Sex differences were also observed in copy number alterations. There were 39 vs. 26 amplifications and 22 vs. 19 deletions among the recurrent focal somatic copy number alterations detected by Genomic Identification of Significant Targets in Cancer (GISTIC) for males vs. females. Graphical representations of significant amplification and deletion events in males and females are shown in Figure 1. Although somatic copy number alterations were more frequently detected and had a stronger signal intensity in males than in females, most of these alterations were co-occurring between males and females, and only a few regions were specific to males or females. Notably, the significant deletion region of 11q22-23 was found only in females, and the amplification signal in this region was also significantly stronger in females than in males. In contrast, the significant amplification region of 16q12-13 was detected only in males. To investigate whether there are sex differences in copy number variations (CNVs) for known or putative bladder cancer-related genes, we compared 20 genes reported in a previous TCGA study and avoided screening for too many candidate genes within the focal peak region. We found that half of the gene regions in the male and female samples showed consistency between the samples, and the other half of the gene regions exhibited some degree of sex specificity. Specifically, four gene regions were prominent in the female sample (RB1, CCNE1, YAP1, and BCL2L1), and six gene regions were highlighted in the male sample (NCOR1, EGFR, ERBB2, MYC, and PTEN). In general, the differences in genes with CNV mutations between men and women are reflected in the height and width of the focal peak signal, as well as the sex specificity of individual repetitive mutation regions.

To verify the sex-specific characteristics of bladder cancer somatic DNA alterations, we have made efforts on the following three aspects: first, we used the somatic mutation data of the non-Caucasian populations in TCGA as validation samples and found that men did have a higher somatic mutation rate than women in bladder cancer across different populations. Second, we examined the mutation rate of bladder cancer-related genes in the, which is the largest comprehensive CSMIC database of cancer-associated somatic mutation sites and does not distinguish by sex. We found that the total mutation rates of the nine sex-specific mutated genes detected in the current study were between the two sex-specific mutation rates, indirectly indicating that the sex-specific mutation rates could exist and be ignored in the overall sample, and thus would be better studied by sex. Finally, by searching for genomic studies related to bladder cancer including both sexes for validation, we found that the only two studies were TCGA studies carried out on bladder cancer twice in succession: one analyzed 131 subjects, and the other expanded the analysis to 412 subjects. The TCGA project is the largest publicly available cancer genome sequencing data project, and it is the gold standard database for cancer genomic research; it is also the source of the data used in the current study. In addition, mutant genes detected in previous TCGA studies have been verified in repeated studies, so the mutant genes detected in current studies through more effective updated software should be credible. Based on the above three aspects, it is reasonable to believe that the results of this study are reliable.

In summary, higher somatic mutation rates were detected for males than for females based on three aspects – category mutation rates by mutation type, cumulative gene mutation rates, and individual mutation rates – suggesting that higher somatic mutation rates may contribute to higher bladder cancer incidence in males. Sex differences were also detected in recurrent somatic gene mutations from exome sequencing data and in somatic copy number alterations from single nucleotide polymorphism (SNP) array data. Interestingly, through the characterization of these discrepancies, four genes (HRAS, NFE2L2, FBXW7, and YAP1) were found to be specifically and significantly mutated in females. We report the first evidence that these four genes are specifically implicated in female bladder cancer patients, which may be associated with the specificity of bladder cancer progression in women. Our study, which was based on somatic DNA alterations, highlighted the sex difference characteristics of bladder cancer, namely, a higher mutation rate among male patients and specific somatic DNA alterations among female patients, which may yield novel insights into the molecular basis of the sex-specific characteristics of bladder cancer.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (No. 81302228) and the Foundation for Outstanding Young Teachers’ Project in Colleges and Universities of Guangdong Province (No. YQ2015033).

Conflicts of interest

None.

References

1. Dobruch J, Daneshmand S, Fisch M, Lotan Y, Noon AP, Resnick MJ, et al. Gender and bladder cancer: a collaborative review of etiology, biology, and outcomes. Eur Urol 2016;69:300–310. doi: 10.1016/j.eururo.2015.08.037.
2. Yuan Y, Liu L, Chen H, Wang Y, Xu Y, Mao H, et al. Comprehensive characterization of molecular differences in cancer between male and female patients. Cancer Cell 2016;29:711–722. doi: 10.1016/j.ccell.2016.04.001.
3. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 2014;507:315–322. doi: 10.1038/nature12963.
4. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell 2018;174:1033. doi: 10.1016/j.cell.2018.07.036.

How to cite this article: Wu TZ, Lei CY, Li JM, Guo YF. Sex discrepancy characterization revealed by somatic DNA alterations in muscle-invasive bladder carcinoma. Chin Med J 2019;132:2492–2494. doi: 10.1097/CM9.0000000000006487

2494