ELSEVIER

Urologic Oncology: Seminars and Original Investigations 37 (2019) 818–825

UROLOGIC ONCOLOGY

Seminars Article

Molecular footprints of muscle-invasive bladder cancer in smoking and nonsmoking patients

Damiano Fantini, Ph.D.[a,b], Roland Seiler, M.D.[c], Joshua J. Meeks, Ph.D.[a,b,d,*]

[a] *Department of Urology, Northwestern University, Feinberg School of Medicine, Chicago, IL*
[b] *Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL*
[c] *Department of Urology, University Hospital Bern, Bern, Switzerland*
[d] *Department of Biochemistry and Molecular Genetics, Northwestern University, Chicago, IL*

Received 6 June 2018; received in revised form 24 August 2018; accepted 3 September 2018

Abstract

Background: Bladder cancer is the fifth most common cancer in the United States and smoking is the largest known risk factor. Tobacco-derived carcinogens may induce the accumulation of somatic mutations in urothelial cells, and likely promote tumorigenesis. However, it is still unknown whether smoking-induced bladder carcinogenesis results in tumors with distinctive molecular features that can be therapeutically exploited.

Methods: We investigated the genomic alterations of human bladder cancer and examined their association with patient smoking history. We performed bioinformatic analyses and looked at differences in gene expression, somatic mutations, and DNA mutational signatures comparing nonsmokers, reformed smokers, and current smokers.

Results: We detected a limited set of gene expression and gene mutation differences between smokers and nonsmokers. We also identified a specific mutational signature that is enriched in tumors from smokers. This mutational signature was described before and has been linked to specific DNA repair defects in human bladder tumors, as well as to the direct effect of nitrosamine carcinogens in the BBN murine model of bladder cancer.

Conclusion: We showed associations between smoking status and selected mutational signatures, which could provide insights in the biology of bladder carcinogenesis and tumor progression. Published by Elsevier Inc.

Keywords: Bladder cancer; Smoking-related carcinogenesis; BBN mouse model; Mutational signatures; APOBEC mutagenesis; Cancer genome

1. Introduction

Bladder cancer is one of the most common cancers in the United States and occurs in men more frequently than in women [1]. About 70% of bladder cancers are nonmuscle invasive, while the remaining patients have muscle invasive (MIBC) or metastatic tumors, with significantly reduced survival [2,3]. Smoking is recognized as the most important risk factor for bladder cancer, and smokers are 4 to 7 times more likely to develop bladder cancer than nonsmokers [4–6]. Tobacco consumption may increase the risk of bladder cancer because of the accumulation of tobacco-derived chemicals in the urine, which in turn cause DNA damage in the urothelium [6,7]. Dietary supplements containing aristolochic acid have also been linked with an increased risk of urothelial cancers, including ureteral and bladder cancer. Aristolochic acid is a carcinogenic, mutagenic, and nephrotoxic phytochemical mainly found in herbal products derived from plants of the *Aristolochia* birthwort family, which are commonly used in Chinese herbal medicine [8,9].

Several studies analyzed the molecular alterations found in bladder cancer genomes [10–13]. These studies revealed MIBC molecular subtypes (such as luminal and basal) similar to those previously characterized in breast cancer [14]. These genomic analyses also demonstrated the

Funding: JJM is supported by Jesse Brown VA Medical Center, Chicago, IL (grant BX003692). DF and JJM are supported by a grant from the John P. Hanson Foundation for Cancer Research (Milwaukee, WI).

*Corresponding author. Tel.: (312)-363-8959.
E-mail address: joshua.meeks@northwestern.edu (J.J. Meeks).

https://doi.org/10.1016/j.urolonc.2018.09.017
1078-1439/Published by Elsevier Inc.

accumulation of *TP53* mutations, as well as mutations affecting epigenetic factors and enzymes involved in chromatin remodeling, highlighting the importance of epigenetic regulation in the biology of bladder cancer [15]. Mutations in DNA repair genes, such as *ERCC2*, were observed and linked to increased cisplatin sensitivity [13,16]. In addition, DNA mutational patterns of bladder cancer were identified [17] that are predominantly comprised of APOBEC mutational signatures [18,19], showing important similarities with other smoking-related cancers, such as lung adenocarcinoma and head and neck cancer.

While these studies contributed to the understanding of molecular alterations promoting MIBC, it is still unknown whether exposure to specific carcinogens, for example, cigarette smoke, results in tumors with distinctive molecular and mutational features that could be clinically or therapeutically exploited. Recent studies revealed that there are no differences in urine markers of smoking and nonsmoking bladder cancer patients [20]. Also, it was found that reformed smokers had a higher risk of developing more aggressive (higher stage) forms of bladder cancer. However, this correlation was not detected in current smokers [7].

Here, we investigated the genomic alterations found in MIBC and their association with the patient smoking history. We documented associations between smoking status and selected mutational signatures, which could provide insights in the biology of bladder carcinogenesis and tumor progression.

2. Materials and methods

Bladder cancer gene expression and mutation data were retrieved from *cBioPortal* (http://www.cbioportal.org/) using R version 3.4.3 (https://www.r-project.org/) and an in-house R package, *TCGAretriever* (https://CRAN.R-project.org/package=TCGAretriever). Smoking status was determined based on the values of the "TOBACCO_SMOKING_HISTORY_INDICATOR" attribute included in the TCGA bladder cancer clinical dataset. RNAseq differential analysis was conducted using the *limma* (https://doi.org/doi:10.18129/B9.bioc.limma) and *edgeR* (https://doi.org/doi:10.18129/B9.bioc.edgeR) bioconductor packages. Heatmaps and tile charts were plotted using the *ggplot2* R package. Subtype calls were generated using previously described models and classifiers [21]. Survival analyses were performed in R, using the *survival* (https://CRAN.R-project.org/package=survival) and the *survminer* (https://CRAN.R-project.org/package=survminer) packages. Mutational signatures were computed by non-negative matrix factorization using *mutSignatures* (version 1.3.7, http://www.mutSignatures.org), as described before [15]. Differentially mutated genes were compared via Fisher exact test. Differences in signature exposures between smoking groups were analyzed by 2-tailed *t* test. Hotspot mutation analysis was conducted as described before [15] and using our *hotspotter* app (http://www.biotechworld.it/bioinf/apps/hotspotter/). The 11 tumors that were found enriched in signature BLCA.6 (relative exposure to BLCA.6 higher than 0.30) were: B77, B89-12, B23, B112, B81-1, TCGA-K4-A6FZ-01, B114, B78, B89-4, B5, and B68. Correlation analysis was performed using the *cor.test()* function in R.

3. Results

3.1. Gene expression in MIBC from smokers and nonsmokers

We analyzed gene expression in the TCGA bladder cancer dataset. This dataset includes detailed information about the smoking status of enrolled patients. Specifically, we found 98 nonsmokers, 177 reformed smokers, and 83 current smokers (Fig. 1A). Nonsmokers are defined as patients who smoked less than 100 cigarettes throughout their life. Patient age was lower in the smoking group (median age: nonsmokers = 69.0; current smokers = 63.5), but no differences were detected in tumor stage and lymph node classification (supplementary Figure 1). We compared gene expression in tumors from current smokers and nonsmokers. More than 18,000 genes were analyzed, but we only detected 1 gene (*GPR15*) that showed significant (fdr-adjusted $P = 8.0e10\text{-}6$) up-regulation in smokers (Fig. 1B). We further analyzed *GPR15* expression across the 3 groups of MIBC patients (nonsmokers, reformed smokers, and current smokers), and found that this gene is overexpressed in a large portion of smokers, but not reformed smokers (Fig. 1C and D). Also, GPR15 was up-regulated in both luminal- and basal-subtype tumors from smokers (supplementary Figure S2). We further analyzed RNAseq data and found that tumors from smokers and nonsmokers had overlapping distribution of molecular cancer subtypes identified by gene expression profiling (supplementary Figure S3). These findings support a model where the smoking status may not be a key determinant of the bladder tumor gene expression profile.

3.2. Gene mutations in bladder tumors from smokers and nonsmokers

We analyzed DNA mutations identified in TCGA tumors from nonsmokers, reformed smokers, and current smokers (Fig. 2A, left). Unlike other smoking-related cancers, such as lung adenocarcinoma (Fig. 2A, right), mutation burden does not track with the smoking status. This observation suggests that even if tobacco-induced DNA mutations may contribute to bladder cancer initiation, other genetic instability processes likely promote further accumulation of DNA mutations in bladder tumors. Interestingly, we recently reported a similar observation from a carcinogen-induced mouse model of bladder cancer [15]. Next, we examined gene mutations (Fig. 2B): *TP53*, *TTN*, *KDM6A*, *MLL2* (*KMT2D*), and *MUC16* were the most frequently mutated genes in all smoking groups, and

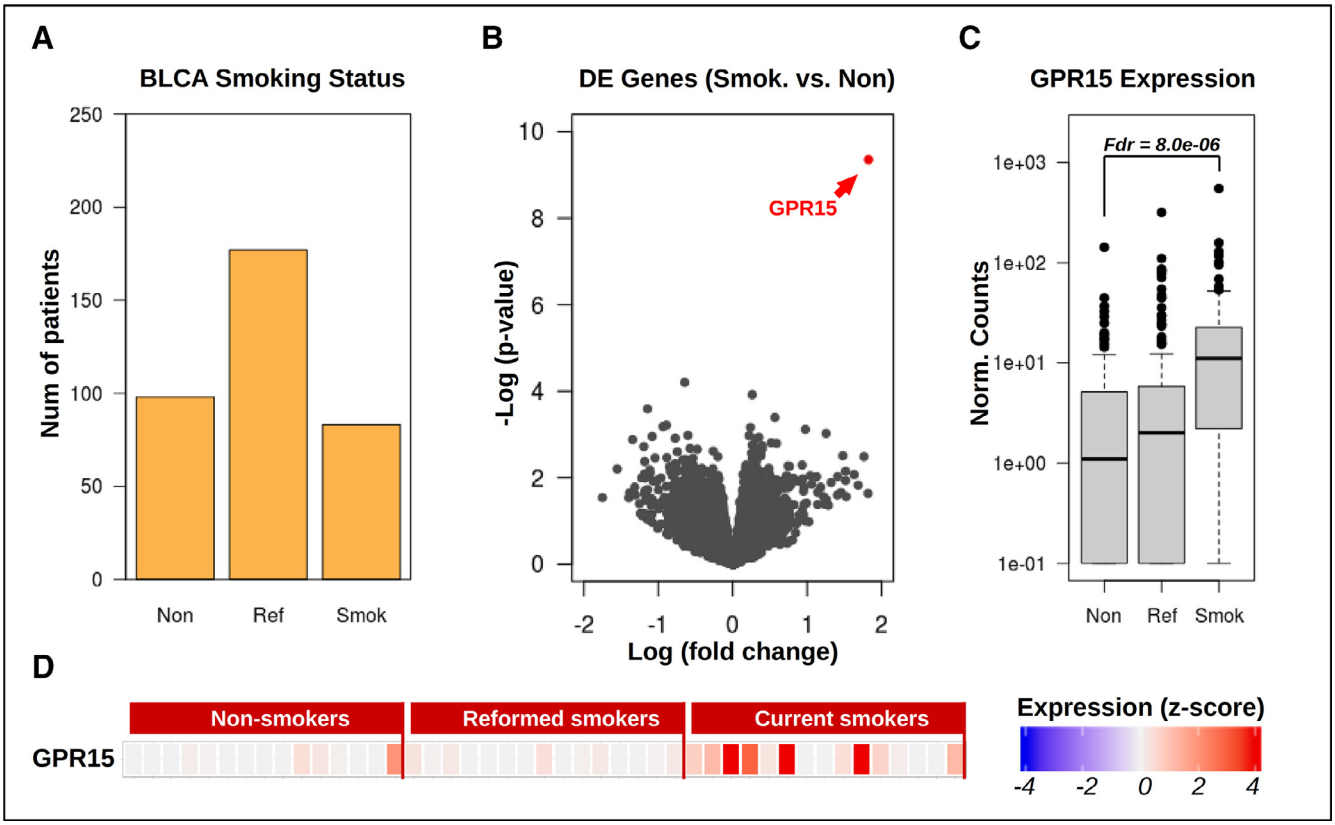

Fig. 1. Gene expression dysregulation in bladder tumors from smokers. (A) Barplot chart summarizing the number of TCGA bladder tumors included in each smoking status group (nonsmokers, *Non*; reformed smokers, *Ref*; current smokers, *Smok*). (B) Volcano plot highlighting genes differentially expressed between nonsmokers and current smokers. The only gene meeting statistically significance criteria (fdr-adjusted $P < 0.01$) is indicated by an arrow. (C, D) Boxplot and heatmap summarizing expression levels (z-scores) of *GPR15* in the 3 tumor groups. In the heatmap, a random sample of 15 tumors from each group was visualized. Red boxes indicate gene overexpression. Color version available online.

accumulated both missense and nonsense mutations (Fig. 2B). Mutation frequency of these genes showed no differences across the 3 groups (Fig. 2C). However, we detected 6 genes with significant differences in mutation frequency between smokers and nonsmokers. *FLG*, *SPTAN1*, *USH2A*, *LYST*, *MED13* were more frequently mutated in tumors from nonsmokers (Fisher $P < 0.05$). *SPTA1* was more frequently mutated in tumors from smokers (Fisher $P = 0.01$). These differences may highlight differential gene mutational patterns in smoking and nonsmoking patients. Nevertheless, none of these genes has a well-established causative role in cancer. We further inspected DNA mutations occurring in the 6 genes by conducting a hotspot mutation analysis. We searched the coding sequences of these genes for regions with distinctive accumulation of DNA mutations in human cancer, which is a typical feature of oncogenes and tumor suppressors (Fig. 2D and E, and supplementary Figure S4). However, we could not detect major hotspot mutations, suggesting that these 6 genes could have a limited role in the process of cancer progression. The observed differences in gene mutation frequency may be the consequence of low overall gene mutation frequency (range: 0.18–0.08) and limited number of patients per group ($n < 100$ per group).

3.3. Extraction of bladder cancer-specific mutational signatures

The interplay between nucleotide context and genetic instability processes plays a crucial role in the accumulation of specific somatic mutations types [17] and results in distinct mutational patterns. DNA mutational signatures were shown to correlate with specific molecular defects found in human and murine tumors [15]. To extract a comprehensive list of mutational signatures active in bladder cancer, we downloaded the most current list ($n = 5$, noncurrent datasets were ignored) of bladder cancer datasets from *cBioPortal* [22]. Mutation burden in these datasets had very different distributions (Fig. 3A), likely because of differences in sequencing technologies and sequencing depths. To analyze mutation data from different datasets together, the *mutSignatures* R package was used, since it enables automatic mutation count normalization. Tumors with at least 30 total mutations per genome were included in the analysis (Fig. 3B), and used for de novo mutational signature extraction. This identified a panel of 6 mutational signatures, similar to those reported before by the Catalogue Of Somatic Mutations In Cancer (COSMIC) [17] initiative from the Sanger Institute (Fig. 3C). Specifically, BLCA.2 and BLCA.3

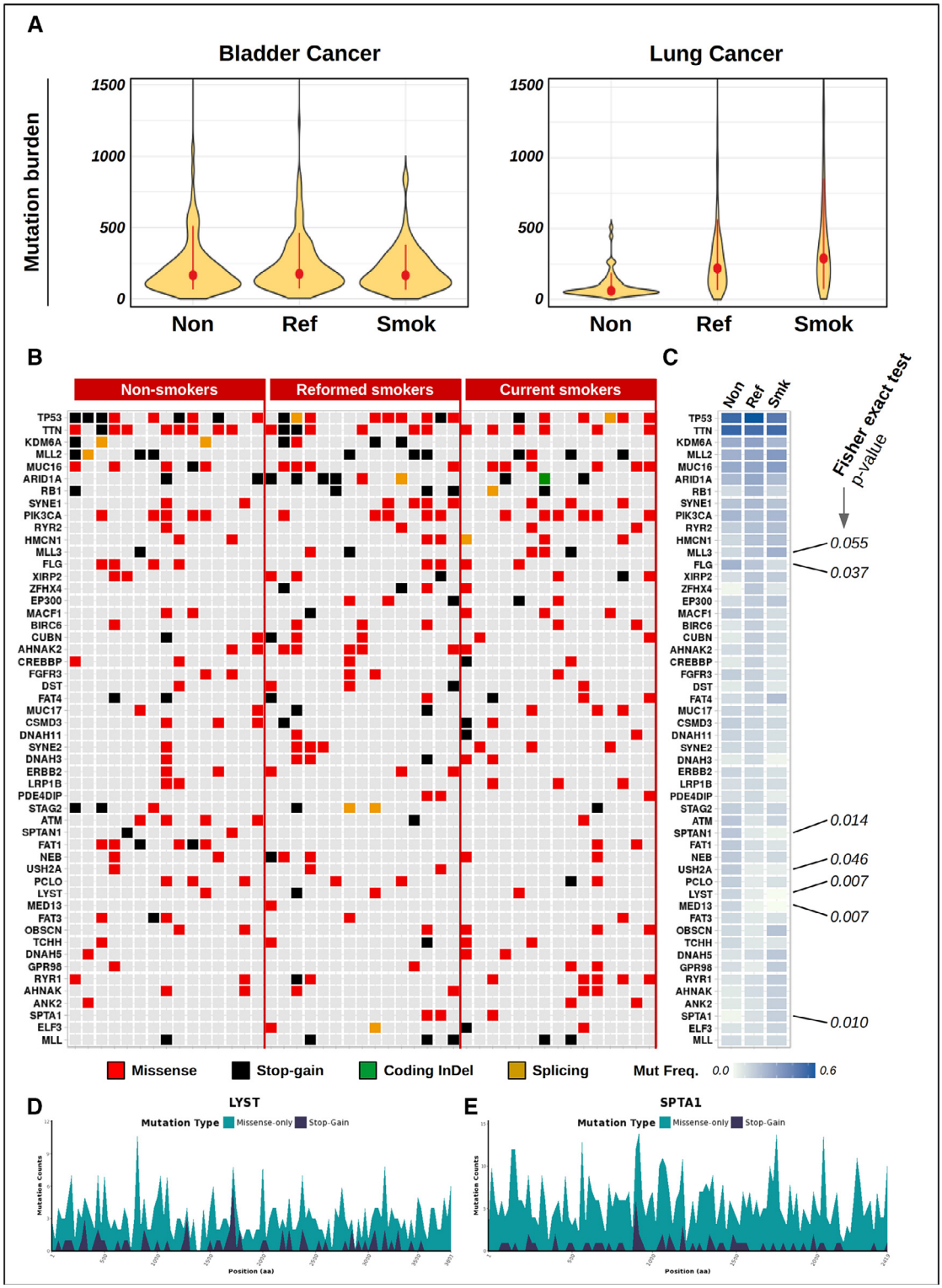

Fig. 2. Gene mutations in bladder tumors from smokers. (A) Violin plots summarizing the distribution of total mutation burden with respect to smoking status in TCGA bladder cancer (left) and TCGA lung adenocarcinoma (right) datasets. Red points and lines indicate the median and the range between the 10th and 90th percentiles. (B) Tile chart showing the types of mutations affecting the most frequently mutated genes in TCGA bladder cancer. Each column corresponds to a different patient. A random sample of 15 tumors from each group was visualized. (C) Heatmap summarizing the ratio of tumors with at last 1 mutation in each of the top mutated genes, segmented according to the patient smoking status. Fisher exact tests were performed to compare nonsmokers and current smokers, and *P* values lower than 0.075 are shown. (D, E) Hotspot mutation analyses for genes *LYST* and *SPTA1*. The plots highlight the distribution of mutations affecting amino acid sequence along the CDS of each gene. Cobalt color indicates stop-gain mutations, cyan indicates missense mutations. Color version available online.

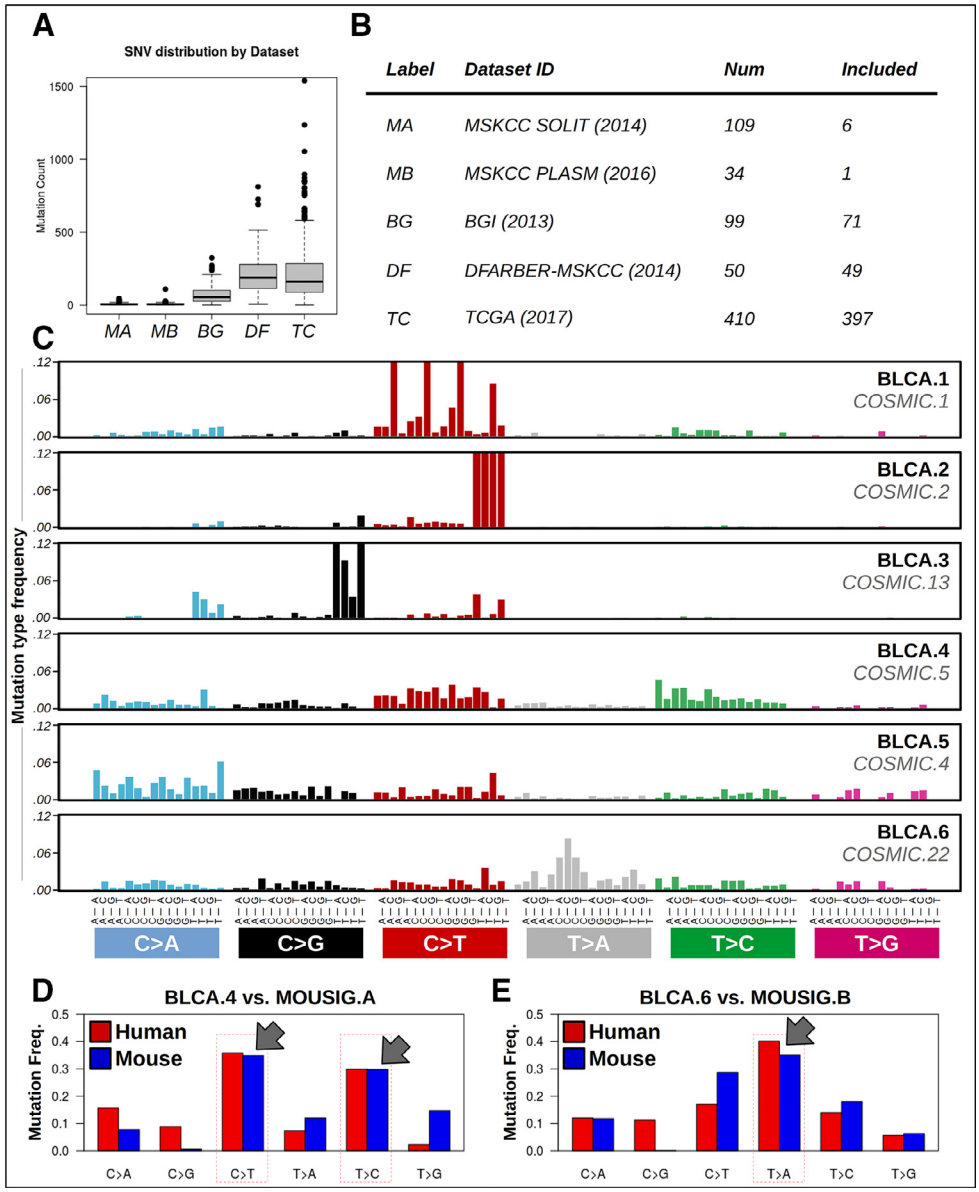

Fig. 3. Mutational signatures identified in bladder cancer. (A) Boxplot summarizing the distribution of mutation burden in samples from different bladder cancer datasets available on *cBioPortal*. (B) Table listing the identifiers of the genomic datasets that were downloaded, and indicating the total number of samples, as well as the number of samples that met inclusion criteria (at least 30 mutations per genome). (C) Barplots illustrating the mutational signatures extracted from bladder cancer. Each bar corresponds to a different trinucleotide mutation type. Bar height indicates relative mutation frequency. The names of the most similar (by cosine similarity) COSMIC signature are reported. (D, E) Barplots comparing single nucleotide variant frequency in the BLCA.4 and MOUSIG.A mutational signatures (D) or in the BLCA.6 and MOUSIG.B signatures (E). Dotted red boxes and arrows indicate the mutations with highest frequency. Color version available online.

matched the APOBEC signatures COSMIC.2 and COSMIC.13. Additionally, our analyses identified signatures BLCA.4 (corresponding to COSMIC.5) and BLCA.6 (corresponding to COSMIC.22) that were similar to those extracted in the BBN-induced urothelial cancer model [15]. This is a bladder cancer mouse model where tumors are induced by prolonged treatment with BBN, a nitrosamine compound related to the carcinogens found in cigarette smoke. Signature BLCA.4 featured high frequency of C > T and T > C transitions (Fig. 3D), similar to the MOUSIG. A signature that was associated with the direct mutagenic effect of the nitrosamine on the urothelium. On the contrary, both BLCA.6 and MOUSIG.B had a distinctive high frequency of T > A transversion (Fig. 3E), which was not due to the activity of the carcinogen, nor to APOBEC mutational processes [15]. Notably, these types of transversions were revealed before in urothelial carcinomas induced by aristolochic acid [8,9], some of which are included in the BGI study [23]. Consistently, 91% (10/11) of the genomes enriched in BLCA.6 mutations came from the BGI dataset.

3.4. Correlation between mutational signatures and smoking history

We further analyzed mutational signature exposures and their correlation with patient's smoking status. Signature exposures are defined as the contribution of each mutational signature to the total number of mutations found in each tumor genome. Mutation signatures BLCA.5 and BLCA.6 were excluded from this analysis since they were detected in a limited number of tumors, and both have median relative exposures lower than 10% (Fig. 4A). We examined TCGA tumor samples with available information about smoking status, and deconvoluted the corresponding trinucleotide mutation counts against signatures BLCA.1-to-4. This analysis (Fig. 4B) confirmed a well-established trend linking mutation load and APOBEC mutagenesis [19], as high mutation burden tracked with elevated exposures to APOBEC signatures (BLCA.2, BLCA.3). A closer analysis of mutational signatures revealed a distinct association between smoking status, BLCA.3, and BLCA.4 (Fig. 4C and D). A number of cancer genomes from smoking patients had elevated signature BLCA.4, and this mutational pattern was significantly increased in tumors from smokers ($P = 0.00038$), suggesting that it may be a direct consequence of the tobacco-derived carcinogens (Fig. 4B and D). On the contrary, exposures to BLCA.3 were higher in tumors from nonsmokers (Fig. 4C, $P = 0.00572$). Consistently, relative exposures to signatures BLCA.3 and BLCA.4 had a strong

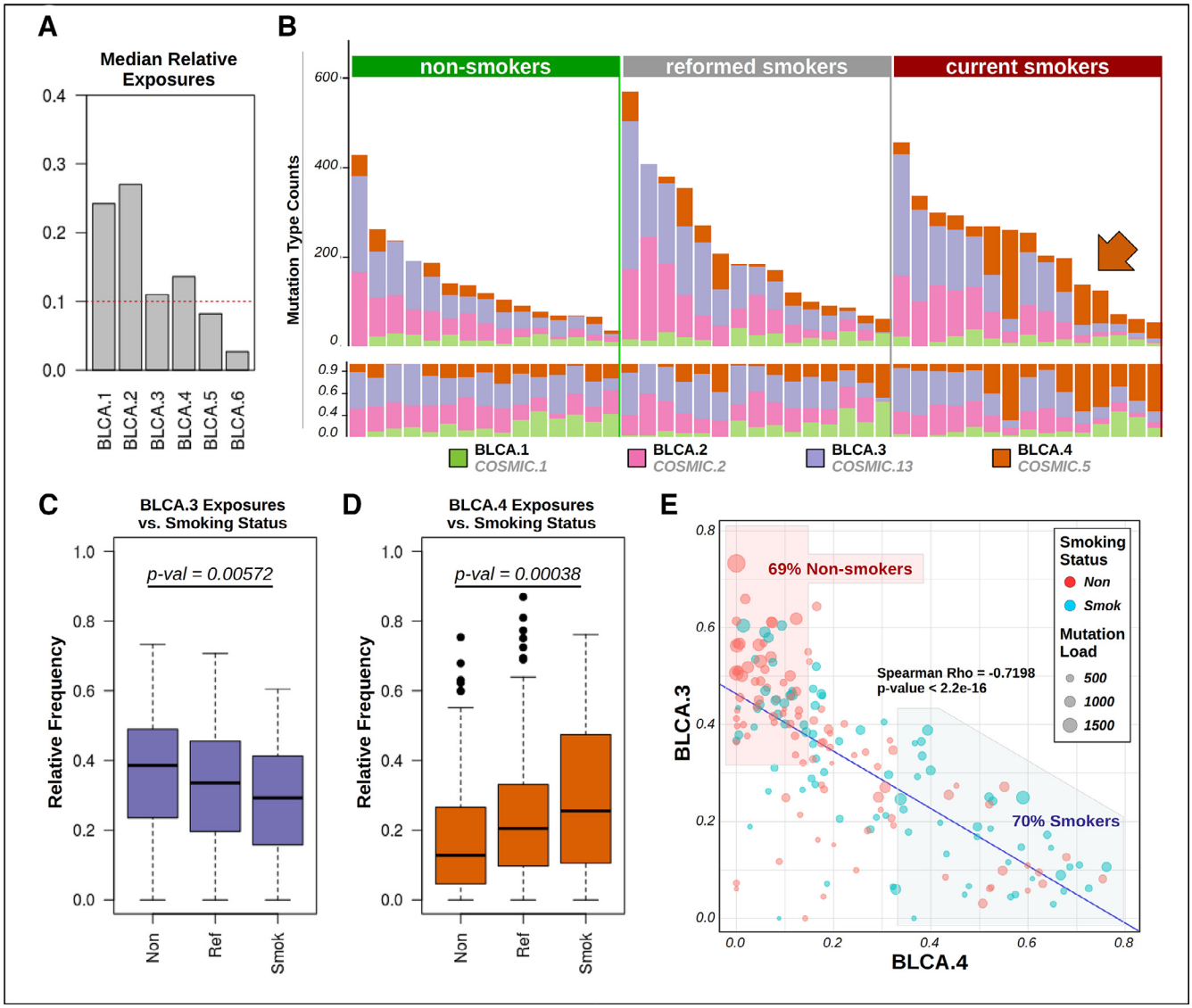

Fig. 4. Correlation between mutational signatures and smoking status in bladder cancer. (A) Barplot showing the median relative exposure of each mutational signature across all analyzed tumor genomes. The dotted red line indicates the threshold at 10%. (B) Absolute (top) and relative (bottom) mutational signature exposures in a random sample (15 samples per group) of bladder cancer tumors. Tumors were grouped by smoking status. A red arrow indicates tumors with high BLCA.4 signature. (C, D) Boxplots summarizing the values of relative exposures to signature BLCA.3 (C) and BLCA.4 (D) in the different tumor groups. (E) Scatterplot displaying all tumors from smokers (cyan) and nonsmokers (red). X-axis and Y-axis indicate relative exposures to signatures BLCA.4 and BLCA.3, respectively. Point size tracks with mutation burden. Color version available online.

inverse correlation (Fig. 4E, Rho = −0.72, $P < 2.2e\text{-}16$). Tumors with elevated levels of BLCA.3 and low BLCA.4 mutations were prevalently found in nonsmoking patients (69%). Conversely, tumors with low BLCA.3 and high BLCA.4 signatures were more frequently found in smokers (70%). This result showed that mutational signatures can provide insights into the molecular processes contributing to bladder tumorigenesis and cancer progression.

4. Discussion

The investigation of genomic aberrations found in tumors, including both expression dysregulation and somatic mutations, can provide valuable insights in cancer research and therapeutics [10,15]. Here, we analyzed TCGA and other genomic datasets of bladder cancer, and examined the relationships among genomic alterations and patient smoking history. Cigarette smoking is a major risk factor for bladder cancer. Tobacco-derived carcinogens may promote DNA lesions in urothelial cells, facilitating transformation and genetic instability. Additionally, nonsmoking bladder cancer patients may have improved survival compared to smokers (supplementary Figure S5).

Analysis of gene expression and gene mutations allowed us to identify only a limited set of differences between smokers and nonsmokers, suggesting that tumors originating as the consequence of tobacco consumption or other causes can progress in a similar fashion, resulting in similar transcriptional defects. Notably, we could not detect a correlation between smoking status and gene expression-derived molecular subtypes of bladder cancer. *GPR15* was the only gene up-regulated in tumors from smokers, and may have a role in tumorigenesis. Consistently, *GPR15* levels were increased in mouse bladder tumors induced by treatment with BBN, a compound that is structurally related to tobacco-derived carcinogens (supplementary Figure S6). Interestingly, *GPR15* was identified as an orphan G protein-coupled receptor expressed by lymphocytes, and was shown to mediate recruitment of effector T cells to inflamed tissue [24]. In addition, recent reports linked *GPR15* and cigarette smoking [25,26]. Since *GPR15* levels were restored to normal upon smoking cessation, this aberration may reflect a specific inflammatory response caused by the activity of tobacco-derived carcinogens on the urothelium. However, it is still unclear whether *GPR15* had a causative role in bladder tumorigenesis.

We also analyzed gene mutations and mutational signatures with respect to patient smoking status. The most commonly mutated genes in MIBC were *TP53*, and the chromatin regulators *KDM6A*, *MLL2* (*KMT2D*), *ARID1A*, and *MLL3* (*KMT2C*). These genes had similar mutation frequency across all tobacco smoking groups. On the contrary, characterization of mutational signatures granted a better segmentation of tumors based on the smoking status. Specifically, we revealed a mutational signature (BLCA.4) that is enriched in tumors from smokers, and corresponds to a mutational signature previously described by the Sanger Institute, namely COSMIC.5 [17]. Interestingly, we recently identified a similar signature (named MOUSIG.A) in murine bladder tumors induced by treatment with BBN, a nitrosamine compound closely related to the carcinogens found in cigarette smoke. In the BBN tumors, mutations associated to this signature are found at comparable levels in all tumors independently on the total mutation burden. Also, MOUSIG.A mutations were the only mutations found in bladders of animals exposed to BBN for only 1 month [15]. These observations are consistent with the COSMIC.5-like mutational pattern being a direct result of the nitrosamine carcinogen. Likewise, in human bladder cancer, COSMIC.5 mutations may be the direct consequence of tobacco-derived carcinogens. Consistently, it was shown that defects in the nucleotide excision repair (NER), the principal pathway responsible for repairing DNA bulky lesions such as those produced by some tobacco-related carcinogens, could promote similar mutational patterns [13]. Notably, NER defects may also affect chromatin regulation, as it was shown that many NER components can regulate gene transcription and chromatin status [27,28].

Unlike other smoking-related tumors such as lung adenocarcinoma, in bladder cancer, mutation burden is independent on the patient smoking history. On the contrary, we confirmed that mutation load is associated with APOBEC-related mutational signatures. These evidences support a model where, depending on the specific gene defect produced by somatic mutations and epigenetic dysregulation, bladder cancer cells may further accumulate DNA mutations via other genetic instability mechanisms, such as APOBEC mutations in humans [18,19], or T > A mutagenesis in both humans and mice [29].

Our study is not without limitations that include the retrospective study design. Moreover, smoking status was assessed using questionnaires. The accuracy of this self-reported smoking exposure is limited [30] and may affect our analysis. We did not investigate the effects of smoking exposure on epigenetic markers. Finally, smoking exposure may only cause nuances on tumor biology that are not depicted using current high throughput techniques.

In conclusion, we showed associations between smoking history and selected mutational signatures, which could provide insights in the biology of bladder carcinogenesis and tumor progression.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgments

DF and JJM designed the research project. DF and RS acquired, prepared, and analyzed the data. DF, RS, and JJM wrote and reviewed the manuscript.

Data availability

A vignette describing the bioinformatic analyses included in this work, as well as the frequency data of all mutational signatures identified by our group and included in the current study (BLCA.1-to-6, and MOUSIG.A-B) are available on GitHub at the following URL: https://github.com/dami82/urol_oncol_2018.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.urolonc.2018.09.017.

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.

[2] Stenzl A, Cowan NC, De Santis M, Kuczyk MA, Merseburger AS, Ribal MJ, et al. Treatment of muscle-invasive and metastatic bladder cancer: update of the EAU guidelines. Eur Urol 2011;59:1009–18.

[3] Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Comperat E, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol 2013;64:639–53.

[4] Leppert JT, Shvarts O, Kawaoka K, Lieberman R, Belldegrun AS, Pantuck AJ. Prevention of bladder cancer: a review. Eur Urol 2006;49:226–34.

[5] Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC. Association between smoking and risk of bladder cancer among men and women. JAMA 2011;306:737–45.

[6] Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, et al. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 2013;63:234–41.

[7] Barbosa ALA, Vermeulen S, Aben KK, Grotenhuis AJ, Vrieling A, Kiemeney LA. Smoking intensity and bladder cancer aggressiveness at diagnosis. PLoS One 2018;13:e0194039.

[8] Rosenquist TA, Grollman AP. Mutational signature of aristolochic acid: clue to the recognition of a global disease. DNA Repair 2016;44:205–11.

[9] Hoang ML, Chen CH, Sidorenko VS, He J, Dickman KG, Yun BH, et al. Mutational signature of aristolochic acid exposure as revealed by whole-exome sequencing. Sci Transl Med 2013;5:197ra102.

[10] Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell 2017;171:540–56:e525.

[11] Al-Ahmadie HA, Iyer G, Lee BH, Scott SN, Mehra R, Bagrodia A, et al. Frequent somatic CDH1 loss-of-function mutations in plasmacytoid variant bladder cancer. Nat Genet 2016;48:356–8.

[12] Kim PH, Cha EK, Sfakianos JP, Iyer G, Zabor EC, Scott SN, et al. Genomic predictors of survival in patients with high-grade urothelial carcinoma of the bladder. Eur Urol 2015;67:198–201.

[13] Kim J, Mouw KW, Polak P, Braunstein LZ, Kamburov A, Kwiatkowski DJ, et al. Somatic ERCC2 mutations are associated with a distinct genomic signature in urothelial tumors. Nat Genet 2016;48:600–6.

[14] Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.

[15] Fantini D, Glaser AP, Rimar KJ, Wang Y, Schipma M, Varghese N, et al. A carcinogen-induced mouse model recapitulates the molecular alterations of human muscle invasive bladder cancer. Oncogene 2018;37:1911–25.

[16] Van Allen EM, Mouw KW, Kim P, Iyer G, Wagle N, Al-Ahmadie H, et al. Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. Cancer Discov 2014;4:1140–53.

[17] Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21.

[18] Glaser AP, Fantini D, Shilatifard A, Schaeffer EM, Meeks JJ. The evolving genomic landscape of urothelial carcinoma. Nat Rev Urol 2017;14:215–29.

[19] Glaser AP, Fantini D, Wang Y, Yu Y, Rimar KJ, Podojil JR, et al. APOBEC-mediated mutagenesis in urothelial carcinoma is associated with improved survival, mutations in DNA damage response genes, and immune response. Oncotarget 2018;9:4537–48.

[20] Deininger S, Hennenlotter J, Rausch S, Docktor K, Neumann E, da Costa IA, et al. No influence of smoking status on the performance of urine markers for the detection of bladder cancer. J Cancer Res Clin Oncol 2018;144:1367–73.

[21] Seiler R, Ashab HAD, Erho N, van Rhijn BWG, Winters B, Douglas J, et al. Impact of molecular subtypes in muscle-invasive bladder cancer on predicting response and survival after neoadjuvant chemotherapy. Eur Urol 2017;72:544–54.

[22] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:l1.

[23] Guo G, Sun X, Chen C, Wu S, Huang P, Li Z, et al. Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation. Nat Genet 2013;45:1459–63.

[24] Suply T, Hannedouche S, Carte N, Li J, Grosshans B, Schaefer M, et al. A natural ligand for the orphan receptor GPR15 modulates lymphocyte recruitment to epithelia. Sci Signal 2017;10:eaal0180.

[25] Bauer M, Fink B, Seyfarth HJ, Wirtz H, Frille A. Tobacco-smoking induced GPR15-expressing T cells in blood do not indicate pulmonary damage. BMC Pulm Med 2017;17:159.

[26] Koks S, Koks G. Activation of GPR15 and its involvement in the biological effects of smoking. Exp Biol Med 2017;242:1207–12.

[27] Fantini D, Huang S, Asara JM, Bagchi S, Raychaudhuri P. Chromatin association of XRCC5/6 in the absence of DNA damage depends on the XPE gene product DDB2. Mol Biol Cell 2017;28:192–200.

[28] Huang S, Fantini D, Merrill BJ, Bagchi S, Guzman G, Raychaudhuri P. DDB2 is a novel regulator of Wnt signaling in colon cancer. Cancer Res 2017;77:6562–75.

[29] Hollstein M, Moriya M, Grollman AP, Olivier M. Analysis of TP53 mutation spectra reveals the fingerprint of the potent environmental carcinogen, aristolochic acid. Mutat Res 2013;753:41–9.

[30] Thong AE, Petruzella S, Orlow I, Zabor EC, Ehdaie B, Ostroff JS, et al. Accuracy of self-reported smoking exposure among bladder cancer patients undergoing surveillance at a tertiary referral center. Eur Urol Focus 2016;2:441–4.