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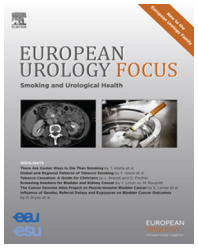

Platinum Priority – Brief Correspondence
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Molecular Characterization of Bladder Cancer in Smokers versus Nonsmokers

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Abstract

Smoking is considered an important risk factor for bladder cancer (BC), yet molecular characterization of BC in nonsmokers has not been extensively studied. Here, we compare molecular differences between smokers and nonsmokers with BC. BC specimens (676) profiled at a Clinical Laboratory Improvement Amendments-certified laboratory from 2006 to 2014 were retrospectively evaluated for molecular differences between smokers and nonsmokers. Protein expression was determined with immunohistochemistry. In situ hybridization was used for *ERBB2* (*HER2/neu*) and *EGFR* evaluation. Genes were evaluated using Sanger or next-generation sequencing. Thirty patients were confirmed lifetime nonsmokers (NS) and 39 were reformed or current smokers (RCS). There was a trend for increased *PIK3CA* mutations in NS versus RCS (43% vs 11%, $p = 0.1760$), whereas *TP53* alterations were higher in RCS versus NS (63% vs 53%, $p = 0.6699$). *EGFR* amplification was observed more in NS versus RCS (22% vs 11%, $p = 0.5815$), while *HER2* was amplified only in RCS (23% vs 0%, $p = 0.05$). The molecular differences between RCS and NS with BC suggest a different oncogenesis with potentially different treatment options.

Patient summary: Bladder cancer patients with no history of smoking have different molecular characteristics than those with smoking history. We found that smokers tend to have higher incidence of *HER2* amplification, whereas nonsmokers seemed to have higher *PIK3CA* mutation. This knowledge provides essential information, which can bear relevance to treatment options.

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Bladder cancer (BC), one of the most common malignancies of the urinary tract [1], accounted for 16 000 cancer-related deaths in 2015 [1]. Smoking is an important risk factor [2], causing half of BC cases. Other risk factors include exposure to aromatic amines, schistosomiasis, and genetic syndromes (eg, germline *Rb1* gene mutation, Cowden, Lynch syndromes, etc.) [3]. Cigarette smoke contains >60 carcinogens that can cause direct DNA damage leading to unique mutational changes in smokers with lung cancer [4]. Studies have shown that smokers with BC have worse cancer specific mortality than nonsmokers [5] and that that smoking exposure negatively affects disease recurrence,

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Table 1 – Demographic details: the table shows the number of bladder cancer patients by age, smoking status, and specimen histology or patient diagnosis

Patient Information			
Cohort	Lifetime NS	Current smoker	Reformed smoker
Total *N*	30	8	31
Median age (yr)	66	65	69
Specimens with staging	14/30	3/8	15/31
0is (Tis)	1	0	0
1	0	0	1
2 (T2)	4	0	1
3 (T3-4a)	5	0	4
4 (T4b or N+/M+)	4	3	7
Specimen Information			
Histology (%)	Lifetime NS		Reformed and current smokers
Urothelial	24 (includes 2 with sarcomatous differentiation)		33 (includes 1 micropapillary)
Nonurothelial	3 (2 adenocarcinoma, 1 neuroendocrine)		3 (1 adenocarcinoma, 2 squamous)
Not otherwise specified	3		3
Location/Site	Lifetime NS		Reformed and current smokers
Primary	24 (includes 8 TURBT, 4 partial or radical cystectomy documented)		30 (includes 7 TURBT, 5 partial or radical cystectomy documented)
Metastatic	5 (4 lymphatic, 1 renal)		9 (4 lymphatic, 2 hepatic, 1 skin, 1 ileocecal, 1 pulmonary, 1 vaginal)

NS = nonsmoker bladder cancers; TURBT = transurethral resection of bladder tumor.

progression, and survival in nonmuscle invasive BC patients [5]. The etiology behind such differences and behind the development of BC in nonsmokers is poorly understood. The Cancer Genome Atlas (TCGA) recently published data detailing the molecular characterization of BC [6], identifying potential therapeutic targets in 69% of tumors, with roughly 42% aberrations identified in the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA)/AKT/mechanistic target of rapamycin pathway and 45% in the receptor tyrosine kinases/mitogen-activated protein kinases pathway [6]. The initial published dataset, comprised of 70% smokers/ex-smokers, identified no significant differences between smokers and never-smokers, although never-smokers had a slightly higher fraction of C>G mutations than smokers [6]. Differences in protein expression by immunohistochemistry (IHC) and gene amplification (in situ hybridization [ISH]) have never been well studied based on smoking status, although this is changing [7,8]. However, with the advent of newer technologies, molecular characterization of BC based on smoking status demands further exploration.

This study was Institutional Review Board exempt. Test results of IHC, ISH, and hot-spot sequencing from BC samples (collected from 2006 to 2014) profiled at a commercial Clinical Laboratory Improvement Amendments-certified laboratory (Caris Life Sciences, Phoenix, AZ, USA) were reviewed. Test methodologies were previously described [7]. Smoking status (nonsmokers [NS]; reformed or current smokers [RCS]), age, and sex were available on a limited number of patients. Fisher's was utilized for statistical analysis.

Of 696 BC cases profiled, smoking history was available on 69 patients; 30 patients were lifetime NS; 39 patients were classified RCS. Reformed smokers were defined as patients who had stopped smoking for more than 5 yr at the time of diagnosis. Patients with detailed past or current smoking history ($n = 9$) averaged 57.8 pack yr (30–120 pack yr). All others had a *long* history of smoking. Table 1 delineates patient cohorts and BC specimens.

In our survey, the NS cohort had a higher rate of PDGFRA expression (30% vs 7.1%, $p = 0.2721$) and loss of PTEN expression versus RCS (50% vs 38.5%, $p = 0.4629$). The RCS cohort was twice as likely to be positive for HER2 expression versus NS (12.8% vs 6.7%, $p = 0.6905$). *EGFR* amplification was seen in 22% of NS versus 11% in RCS ($p = 0.5815$), whereas *HER2* was exclusively amplified in RCS (2.7% vs 0%,

Table 2 – Amplification or rearrangement by fluorescence or chromogenic in situ hybridization (*HER2* was exclusively amplified in thr RCS cohort, $p = 0.05$)

	Overall			NS			RCS		
Biomarker	Amplified	Total	Percent	Amplified	Total	Percent	Amplified	Total	Percent
MET	2	221	0.9	0	11	0.0	0	7	0.0
EGFR	47	217	21.7	2	9	22.2	2	18	11.1
ERBB2 (HER2)	44	444	10.3	0	17	0.0	5	22	22.7
TOP2A	3	56	5.4	0	1	0.0	0	4	0.0

EGFR = epidermal growth factor receptor; *HER2* = human epidermal growth factor receptor 2; *MET* = MET proto-oncogene; M = metastases; N = node; NS = nonsmoker bladder cancers; *TOP2A* = topoisomerase II alpha; T = tumor; RCS = reformed or current smokers.

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