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Age at Diagnosis, Obesity, Smoking, and Molecular Subtypes in Muscle Invasive Bladder Cancer

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Abstract

Background—Heterogeneity of muscle-invasive bladder cancer (MIBC) has been characterized using whole genome mRNA expression data, showing distinct molecular and clinicopathological characteristics by subtypes. However, associations between risk factors and molecular subtypes have not been reported.

Methods—Four previously published schemes were used to categorize molecular subtypes in 372 MIBC patients from the Cancer Genome Atlas (TCGA). Data on gene expression (RNA-seq), demographic, and clinicopathological characteristics were retrieved through TCGA data portal. Polytomous logistic regression was used to estimate the associations of subtypes by different schema with age at diagnosis, obesity, and smoking.

Results—While some quantitative variation was evident, distinct molecular subtype schemes showed considerable consistency in the association with the risk factors. Generally, compared to patients with luminal-like tumors, patients with basal-like subtypes were more likely to be older ($OR_{75+yrs\ vs.\ <60\ yrs}$ range=1.32–2.89), obese ($OR_{obese\ vs.\ normal}$ range=1.30–3.05), and to start smoking at early age ($OR_{<18\ yrs\ vs.\ 25+\ yrs}$ range=1.11–4.57).

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Conflict of interest statement None declared

Conclusions—Different molecular subtypes of MIBC may have distinct risk profiles. Large population-based studies with detailed information on bladder cancer risk factors are needed to further define etiologic heterogeneity for bladder cancer.

Keywords

muscle-invasive bladder cancer; molecular subtype; age; obesity; smoking

Background

Recently, molecular heterogeneity of muscle-invasive bladder cancer (MIBC) has been characterized using whole genome mRNA expression data (1–4). There are two to five molecular subtypes reported by different investigators: at Lund University (Lund: urothelial-like A, genomically unstable, infiltrated, urothelial-like B, and squamous cell carcinoma-like), MD Anderson (MDA: basal, luminal, and p53-like), the Cancer Genome Atlas (TCGA: Cluster I-IV), and our institution (UNC: basal and luminal) (1–5). These molecular schemes have considerable similarities (e.g. the existence of basal/squamous-like subtype), and all schemes show some value in predicting progression (6). However, the etiologic relevance of the molecular subtypes is unknown.

We hypothesized that different mechanistic pathways are involved in the development and progression of different MIBC subtypes, and therefore, that these subtypes may differ in associations with risk factors. To test this hypothesis, we classified 372 MIBCs from TCGA data using the four published subtyping schemes (Lund, MDA, TCGA, and UNC), and then evaluated the subtype-specific associations of each scheme with age at diagnosis, obesity, and cigarette smoking.

Methods

The study population included 372 MIBCs patients from TCGA with available molecular subtype data by UNC-BASE47 (4). Data on gene expression (RNA-seq), demographic, and clinicopathological characteristics were retrieved through TCGA data portal (https://tcga-data.nci.nih.gov/tcga/). MDA and Lund subtype calls were directly extracted from the results of previous published literatures (1–3, 5). TCGA subtypes of 129 MIBCs were extracted from the original published paper (2). The rest 241 cases were classified using the ClaNC method with the nearest centroid classifier estimated based on the silhouette width for the 129 cases and the 2,708 genes in the original paper (7, 8). All of the patients had provided informed consent to TCGA. Protocols were reviewed by the Institutional Review Board at all participating institutions.

Information of demographic, anthropometric, and clinicopathologic characteristics in TCGA data base was extracted from clinical records (https://tcga-data.nci.nih.gov/tcga/; file name: nationwidechildrens.org_clinical_patient_blca). Weight and height were used to calculate body mass index (BMI). In the analyses, age at diagnosis and BMI were used both continuously and categorically (age, <60 years, 60–<75 years, or ≥ 75 years; BMI, normal weight as <25kg/m^2, overweight as 25-<30kg/m^2, or obese as ≥30kg/m^2). Never smokers were defined as patients who were not smoking at the time of interview and had smoked less

than 100 cigarettes in their lifetime. Former smokers were defined as patients who were not smoking at the time of interview but had smoked at least 100 cigarettes in their lifetime. Current smokers were defined as patients who were daily or occasionally smoking at the time of interview. Ever smokers (former and current smokers) were asked about their smoking history, including age at smoking initiation, and the beginning and end of smoking period along with the frequency and intensity of smoking. Smoking variables in this study were defined as smoking status (never, former, or current), age at smoking initiation (<18 years, 18-<25 years, or ≥25 years), total smoking years (<17 years, 17-<40 years, or ≥ 40 years), total pack-years (<20 years, 20–40 years, or ≥ 40 years) among ever smokers, and quitting recency (time since quitting <10 years or ≥ 10 years) among former smokers.

The differences in variable distribution were assessed using ANOVA F-tests for continuous variables, and χ^2 test or Fisher's exact test for categorical variables. Polytomous logistic regression was used to calculate unadjusted odds ratios (ORs), adjusted ORs, and the corresponding 95% confidence intervals (CIs). Overall association heterogeneity was estimated by Wald χ^2 test (9). To account for potential confounders, associations with obesity and smoking were adjusted for age. Associations with obesity were additionally adjusted for smoking status. Associations with age at smoking initiation were additionally adjusted for total smoking years and pack-years, given previously reported correlation between these variables (10, 11) and the observation that smoking initiation was associated with both variables in TCGA (total smoking years, χ^2 test p=0.04; pack-years, χ^2 test p <0.01). All statistical tests were two-sided with α of 0.05. All analyses were performed using SAS version 9.2 (SAS Institute).

Results

Molecular subtypes by different schemes

To facilitate the comparison across different schemes, we used the simplest two-subtype UNC scheme (UNC luminal vs UNC basal) as the primary scheme. Its distribution by clinicopathological features and subtypes defined by other schemes is shown in Supplementary Table 1. Consistent with previous analyses, UNC luminal subtype was associated with low-grade and early-stage papillary tumors (p<0.01). Subtypes defined by the other schemes showed considerable overlap (Figure 1 and Supplementary Table 1). The majority of UNC luminal tumors were classified as Lund urothelial-like A/genomically unstable (91%), MDA luminal (75%), and TCGA Cluster I (64%), while the majority of UNC basal tumors were classified as Lund infiltrated/urothelial-like B/SCC-like (91%), and MDA basal (79%). Almost all of squamous-like tumors (TCGA Cluster III, 98%; Lund SCC-like, 100%) were classified as UNC basal. 89% of TCGA Cluster IV, which has been hypothesized to correspond to claudin-low subtype (12, 13) were classified as UNC basal. MDA p53-like did not show a significant correlation with UNC subtypes, with an almost even distribution of UNC basal and luminal subtypes in our analysis (χ^2 test p=0.41). This is an interesting observation because this subtype was previously proposed as a subset of UNC luminal (1, 12).

Considering the overlap and similarity across different schemes, for simplicity and to ensure statistical power in the following analyses we named UNC luminal, TCGA Cluster I&II,

MDA luminal, and Lund urothelial-like A/genomically unstable as “luminal-like” tumors; and named UNC basal, TCGA Cluster III&IV, MDA basal, and Lund infiltrated/urothelial-like B/SCC-like as “basal-like” tumors.

Associations with age at diagnosis, obesity, and smoking

Luminal-like and basal-like tumors had unique risk factor associations. Generally, older (≥60 years) and overweight/obese patients were more likely to have basal-like tumors across all subtype schemes, although not all schemes showed statistically significant associations (Table 1). The associations with obesity were independent of age. The magnitude of the association of subtype with obesity was slightly stronger for MDA and Lund schemes. Additional adjustment for smoking status did not substantially change the measures of the obesity-subtype association, except to decrease precision (Supplementary Table 2). On a continuous age scale, only MDA p53-like showed a significant association with age, with the odds increasing 0.06 (OR=1.06, 95% CI=1.02–1.10) for each one-year increment in age.

The associations between subtypes and cigarette smoking are shown in Table 2. Compared with luminal-like cases, basal-like cases tended to be former smokers, but none of the associations were statistically significant. Among ever smokers, patients starting smoking at earlier ages were significantly more likely to have basal-like subtypes. These associations were independent of age, but varied slightly for different schema, with stronger associations observed in MDA and Lund schemes. To account for the potential confounding effect by total smoking years and pack-years, we estimated ORs additionally adjusted for the two variables (Supplementary Table 2), with no significant changes observed in the magnitude of ORs. None of the following variables: time since quitting, total smoking years and pack-years showed significant associations with subtypes by different schemes (Supplementary Table 3), while former smokers quitting ≥ 10 years and smokers with pack-years ≥ 40 were suggested to have higher risk for basal-like tumors.

Discussion

In this study we mapped the molecular subtypes by different schemes on 372 MIBCs patients from TCGA, and linked molecular subtypes to three of the most important risk factors of bladder cancer. In line with previous biological studies (1, 6, 12) where biological and clinicopathological associations with molecular subtypes have been similar across different schemes, we observed consistent associations with etiologic exposures. In general, patients with basal-like subtypes were more likely to be obese, diagnosed at advanced age, and to start smoking at an early age. However, etiologic associations appeared slightly stronger with the subtypes defined by MDA and Lund schemes.

Currently, there are no data describing the associations of risk factors with MIBC transcriptional subtype, although differences by subtypes defined by clinicopathlogical features have been reported (14–18). Considering that basal-like tumors tend to be higher grade and stage than luminal-like tumors, our findings are in line with the previous studies, in which smokers, obese and older patients have been consistently found to have higher stage and grade (14–18). Although earlier age of smoking initiation is associated with higher

risk for bladder cancer, its influence on pathological features of bladder cancer has received less investigation (19).

Biologic and genomic data already suggest that bladder cancers may develop and progress through distinct pathways, and the current analysis highlights a plausible role of risk factor exposures in initiating or promoting these pathways. These findings can provide important novel clues with public health relevance. For instance, we found age at smoking initiation was the strongest factor that may drive MIBC heterogeneity, and associated with basal-like subtype. This result suggests exposure of smoking in an early “susceptible window” may play a role at the early phase of tumorigenesis of basal-like tumors. Although pathways specifically linking early smoking initiation remain understudied, this hypothesis is supported by the recent studies of temporal tumor heterogeneity in non-small cell lung cancer, where smoking-related genomic events represent many of very early mutations in tumors (20, 21).

The heterogeneity in the association of bladder cancer with risk factors also provides novel explanations for some unresolved findings in epidemiological studies. For instance, given the heterogeneous relationship of obesity and MIBC subtype, the variation in subtype composition of study populations could contribute to the observed inconsistent findings in the association between obesity and MIBC (22, 23). Furthermore, relatively strong association of basal-like subtype with advanced age and smoking provides another interpretation for the increasing smoking-associated risk of bladder cancer observed since 1990s (24–27), as obesity epidemic and population aging in the past decades potentially increased proportion of basal-like tumors in bladder cancer cases, which artificially could shift up the association between smoking and bladder cancer as a whole.

In the past decade, genetic, genomic and epigenetic analyses provide ample evidence for enormous diversity in tumor molecular features, and based on these features some cancers have been classified into several molecular subtypes. Recent analysis revealed that many molecular alterations are shared by certain subtype across cancers arising from different origins (e.g. basal subtype in breast and bladder cancers), suggesting common oncogenic pathways and similarities in cell type origins (e.g. urothelial basal cells and breast basal cells) (4, 28). In risk factor analyses, common risk factors are also observed in similar subtypes of different cancers, such as obesity and basal subtype in breast cancer and bladder cancer. Interestingly in this study we found age is positively associated with basal-like MIBC, which is on the opposite direction of the association with basal breast cancer. This distinct effect of age on the two diseases is reflected by their difference in age distribution, where age distribution of breast cancer follows a bimodal pattern with different intrinsic subtype enriched in different age groups (29), while bladder cancer shows an unimodal pattern with highest rate observed among people aged 75–84 years (30). Likely these differences result from differences in tissue/organ of origin, etiology determined by exposome (a totality of exposure, e.g. hormone) and host characteristics. However, current data from both biological and epidemiological studies are still not enough to explain the similarities and differences in the subtype-specific etiology.

Our analysis has some limitations. This study used TCGA data, which is not originally designed to study risk factors. Consequently, the completeness and accuracy of information on risk factors are lower than well-designed epidemiological studies or clinical trials. For instance, in our analysis the missing percentage of BMI was 13%, which may influence the association estimates quantitatively, although less likely substantially. Moreover, TCGA data includes a relatively low number of female, non-white cases, and non-muscle invasive cases. Therefore we cannot estimate the associations among these subpopulations, although associations of bladder subtype with invasive status, gender and race have been suggested (1, 4, 31–33). At last, our study sample size is small, leading to imprecise effect estimates (e.g. ORs of MDA p53-like). However, our results suggest that further research to evaluate etiologic heterogeneity may reveal complexities in associations for established risk factors, and may help to identify novel risk factors and public health strategies for bladder cancer prevention.

Conclusion

In conclusion, our analyses enhance the understanding in etiological heterogeneity of MIBC by integrating molecular biology and risk factor data, showing that different molecular subtypes may have distinct risk profiles. Large population-based studies with detailed information on bladder cancer risk factors are needed to overcome the limitations of our study and validate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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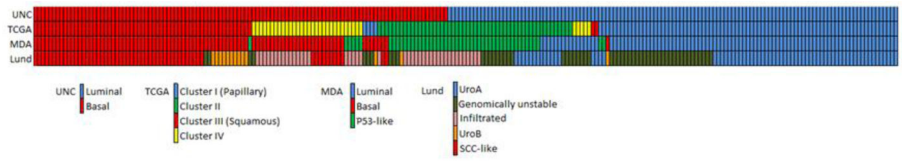

Figure 1.

Associations among molecular subtype schemes*.

*Based on complete data.

Table 1

The association of intrinsic subtypes with age and obesity*.

Molecular subtype	Age at diagnosis			Obesity†		
	<60 years (n=77)	60–<75 years (n=168)	75+ years (n=127)	Normal (n=142)	Overweight (n=108)	Obese (n=74)
UNC						
Basal	31 (40.26)	90 (53.57)	70 (55.12)	61 (42.96)	61 (56.48)	38 (51.35)
Luminal (ref)	46 (59.74)	78 (46.43)	57 (44.88)	81 (57.04)	47 (43.52)	36 (48.65)
OR	1	1.71 (0.99, 2.96)	1.82 (1.03, 3.24)	1	1.61 (0.96, 2.69)	1.42 (0.80, 2.52)
p-value		0.09			0.17	
TCGA						
Cluster III&IV	26 (33.77)	70 (41.67)	51 (40.16)	49 (34.51)	44 (40.74)	30 (40.54)
Cluster I&II (ref)	51 (66.23)	98 (58.33)	76 (59.84)	93 (65.49)	64 (59.26)	44 (59.46)
OR	1	1.41 (0.80, 2.46)	1.32 (0.73, 2.38)	1	1.22 (0.72, 2.06)	1.30 (0.72, 2.32)
p-value		0.50			0.62	
MDA						
Basal	15 (33.33)	35 (38.04)	35 (45.05)	24 (31.17)	25 (42.37)	17 (43.59)
p53-like	4 (8.89)	24 (26.09)	20 (25.32)	14 (18.18)	12 (20.34)	12 (30.77)
Luminal (ref)	26 (57.78)	33 (35.87)	21 (27.63)	39 (50.65)	22 (37.29)	10 (25,64)
OR_{basal}	1	1.84 (0.83, 4.07)	2.89 (1.25, 6.66)	1	1.77 (0.80, 3.91)	3.05 (1.15, 8.08)
OR_{p53}	1	4.73 (1.46, 15.33)	6.19 (1.83, 20.92)	1	1.43 (0.55, 3.76)	3.23 (1.08, 9.66)
p-value		0.02			0.14	
Lund						
Infiltrated/UroB/SCC-like	17 (37.78)	47 (51.09)	43 (56.58)	31 (40.26)	30 (50.85)	22 (56.41)
Genomically unstable	12 (26.67)	18 (19.57)	15 (19.74)	14 (18.18)	17 (28.81)	8 (20.51)
UroA (ref)	16 (35.56)	27 (29.35)	18 (23.68)	32 (41.56)	12 (20.34)	9 (23.08)
$cOR_{Inf/UB/SCCL}$	1	1.64 (0.71, 3.76)	2.25 (0.94, 5.40)	1	2.65 (1.13, 6.22)	2.65 (1.01, 6.91)
OR_{GU}	1	0.89 (0.34, 2.32)	1.11 (0.40, 3.07)	1	3.48 (1.29, 9.36)	2.26 (0.70, 7.29)
p-value		0.38			0.05	

*Total numbers slightly varied due to missing data. P-values of Wald χ^2 test indicated the statistically significance of overall heterogeneity.

†ORs of obesity were adjusted for age.

Table 2

The association of intrinsic subtypes with smoking status and age starting smoking *.

Molecular subtype	Smoking status			Age starting smoking among ever smokers		
	Never (n=104)	Current (n=77)	Former (n=179)	≥25 years (n=38)	18–<25 years (n=57)	<18 years (n=161)
UNC						
Basal	49 (47.16)	34 (44.16)	101 (56.42)	15 (39.47)	29 (50.88)	91 (56.52)
Luminal (ref)	55 (52.88)	43 (55.84)	78 (43.58)	23 (60.53)	28 (49.12)	70 (43.48)
Age-adjusted OR	1	0.89 (0.49, 1.64)	1.36 (0.83, 2.23)	1	1.78 (0.76, 4.16)	2.30 (1.09, 4.82)
p-value		0.2564			0.0847	
TCGA						
Cluster III&IV	38 (36.54)	27 (35.06)	76 (42.46)	15 (39.47)	23 (40.35)	65 (40.37)
Cluster I&II (ref)	66 (63.46)	50 (64.94)	103 (57.54)	23 (60.53)	34 (59.65)	96 (59.63)
Age-adjusted OR	1	0.91 (0.48, 1.70)	1.20 (0.72, 1.98)	1	1.09 (0.47, 2.55)	1.11 (0.53, 2.31)
p-value		0.5847			0.9629	
MDA						
Basal	19 (33.93)	13 (29.55)	48 (46.15)	7 (30.43)	9 (29.03)	45 (47.27)
p53-like	15 (26.79)	10 (22.73)	22 (21.15)	2 (8.70)	7 (22.58)	23 (24.47)
Luminal (ref)	22 (39.29)	21 (47.73)	34 (32.69)	14 (60.87)	15 (48.39)	26 (27.66)
Age-adjusted OR_{basal}	1	0.81 (0.31, 2.13)	1.47 (0.68, 3.20)	1	1.27 (0.35, 4.68)	3.64 (1.24, 10.69)
Age-adjusted OR_{p53}	1	0.91 (0.31, 2.65)	0.83 (0.34, 1.99)	1	3.23 (0.53, 19.65)	6.11 (1.20, 31.14)
p-value		0.4725			0.0344	
Lund						
Infiltrated/UroB/SCC-like	29 (51.79)	15 (34.09)	58 (55.77)	7 (30.43)	13 (41.94)	53 (56.38)
Genomically unstable	8 (14.29)	12 (27.27)	22 (21.15)	4 (17.39)	8 (25.81)	22 (23.40)
UroA (ref)	19 (33.93)	17 (38.64)	24 (23.08)	12 (52.17)	10 (32.26)	19 (20.21)
Age-adjusted $OR_{Inf/UB/SCCL}$	1	0.63 (0.25, 1.59)	1.48 (0.69, 3.16)	1	2.11 (0.58, 7.68)	4.57 (1.53, 13.65)
Age-adjusted OR_{GU}	1	1.80 (0.58, 5.60)	2.29 (0.83, 6.35)	1	2.41 (0.52, 11.04)	3.64 (0.97, 13.68)
p-value		0.1803			0.0567	

* Total numbers slightly varied due to missing data. P-values of Wald χ^2 test indicated the statistically significance of overall heterogeneity.