EBioMedicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Meta-Analysis of the Luminal and Basal Subtypes of Bladder Cancer and the Identification of Signature Immunohistochemical Markers for Clinical Use

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 16 August 2016
Accepted 23 August 2016
Available online xxxx

Keywords:
Bladder cancer
Biomarker

ABSTRACT

Background: It has been suggested that bladder cancer can be divided into two molecular subtypes referred to as luminal and basal with distinct clinical behaviors and sensitivities to chemotherapy. We aimed to validate these subtypes in several clinical cohorts and identify signature immunohistochemical markers that would permit simple and cost-effective classification of the disease in primary care centers.

Methods: We analyzed genomic expression profiles of bladder cancer in three cohorts of fresh frozen tumor samples: MD Anderson (n = 132), Lund (n = 308), and The Cancer Genome Atlas (TCGA) (n = 408) to validate the expression signatures of luminal and basal subtypes and relate them to clinical follow-up data. We also used an MD Anderson cohort of archival bladder tumor samples (n = 89) and a parallel tissue microarray to identify immunohistochemical markers that permitted the molecular classification of bladder cancer.

Findings: Bladder cancers could be assigned to two candidate intrinsic molecular subtypes referred to here as luminal and basal in all of the datasets analyzed. Luminal tumors were characterized by the expression signature similar to the intermediate/superficial layers of normal urothelium. They showed the upregulation of PPARγ target genes and the enrichment for FGFR3, ELF3, CDKN1A, and TSC1 mutations. In addition, luminal tumors were characterized by the overexpression of E-Cadherin, HER2/3, Rab-25, and Src. Basal tumors showed the expression signature similar to the basal layer of normal urothelium. They showed the upregulation of p63 target genes, the enrichment for TP53 and RB1 mutations, and overexpression of CD49, Cyclin B1, and EGFR. Survival analyses showed that the muscle-invasive basal bladder cancers were more aggressive when compared to luminal cancers. The immunohistochemical expressions of only two markers, luminal (GATA3) and basal (KRT5/6), were sufficient to identify the molecular subtypes of bladder cancer with over 90% accuracy.

Interpretation: The molecular subtypes of bladder cancer have distinct clinical behaviors and sensitivities to chemotherapy, and a simple two-marker immunohistochemical classifier can be used for prognostic and therapeutic stratification.

Funding: U.S. National Cancer Institute and National Institute of Health.

1. Introduction

Recent genomic investigations of bladder cancer have revealed complex alterations with heavy mutational load and frequent involvement of chromatin remodeling genes (Cancer Genome Atlas Research N, 2014; Gui et al., 2011; Lawrence et al., 2013). Other studies have identified distinct genomic signatures associated with cancer progression, metastasis and response to therapeutic manipulations (Takata et al., 2005; Puzio-Kuter et al., 2009; Cheng et al., 2013; Van Allen et al., 2014; Groenendijk et al., 2016; Dyrskjot et al., 2003). Several groups used whole genome expression profiling to classify bladder cancer into various distinct subtypes (Cancer Genome Atlas Research N, 2014; Damrauer et al., 2014; Choi et al., 2014a; Lindgren et al., 2010; Sjodahl et al., 2012). Although the names for the respective classes used by these groups were different, they showed striking similarities to the intrinsic basal and luminal subtypes identified in human breast cancers (Damrauer et al.,

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http://dx.doi.org/10.1016/j.ebiom.2016.08.036

2014; Choi et al., 2014b; Perou et al., 2000). In general, the markers that are used to classify bladder cancers into the two major groups reflect an expression signature of normal basal and intermediate/luminal urothelial cell layers (Choi et al., 2014b). Most importantly, the two intrinsic subtypes of bladder cancer show distinct clinical behaviors and responses to frontline chemotherapy (Choi et al., 2014a; Choi et al., 2014b; McConkey et al., 2015). In the chemotherapy naive setting, the muscle-invasive bladder cancers of the basal subtype were more aggressive with shorter survival when compared to luminal cancers (Choi et al., 2014a; Choi et al., 2014b). On the other hand, basal bladder cancers were more sensitive to cisplatinum based chemotherapy and the patients with this form of the disease appeared to gain more benefits from frontline chemotherapy when compared to luminal subtypes (Choi et al., 2014a; Choi et al., 2014b).

Since the classification of bladder cancer into intrinsic molecular subtypes provides prognostic information and may help to identify a subgroup of patients with increased sensitivity to chemotherapy, we performed a meta-analysis of the luminal and basal subtypes of bladder cancer in several MD Anderson and publicly available cohorts. We also validated the signature profiles of luminal and basal cancers on retrospectively collected paraffin-embedded tumor samples, as these are the types of tissue on which the standard of clinical care is based. Finally, in order to identify a minimal set of clinically applicable biomarkers permitting simple classification of bladder cancers into luminal and basal subtypes, we performed image assisted analysis of selected immunohistochemical markers on parallel tissue microarrays.

2. Methods

2.1. Patients and Study Design

The expression profiling studies of molecular subtypes of bladder cancer were conducted on four cohorts: (Cancer Genome Atlas Research N, 2014) the MD Anderson cohort of fresh frozen bladder tumor tissue (n = 132); (Gui et al., 2011) the cohort of fresh frozen bladder tumor tissue from Lund University in Sweden (n = 308) referred to as the Lund cohort; (Lawrence et al., 2013) the Cancer Genome Atlas (TCGA) cohort of fresh frozen bladder tumor tissue (n = 408); and (Takata et al., 2005) the MD Anderson cohort of formalin-fixed paraffin-embedded bladder tumor tissue samples (n = 89) (Table 1).

The MD Anderson cohort of fresh frozen bladder tumor samples was from 100 men and 32 women (mean age 67.2 years ± 12.3 SD). The tumors were classified according to the World Health Organization histologic grading system into low-grade (n = 25) and high-grade (n = 107) (Moch et al., 2016). According to the TNM staging system the tumors were divided into superficial (stage Ta-Tis; n = 34) and invasive (stage T1and higher; n = 98) categories (Sobin et al., 2009).

The Lund cohort mRNA expression and clinical data were retrieved from GEO (GSE32894) as per the original publication (Sjodahl et al., 2012). This cohort consisted of fresh frozen bladder tumor tissue samples from 80 women and 228 men (mean age 70.6 years ± 11.8 SD). The tumors were divided into non-invasive (Ta and Tis; n = 116) and invasive (T1 and higher; n = 190) according to the TNM staging system. The tumors were considered low-grade (n = 151) if they were originally reported as grade 1–2 and high-grade (n = 155) if they were

Table 1
Summary of clinical data: the MD Anderson, TCGA[#], Lund,[*] and FFPE MD Anderson cohorts.

Stage	Subtype	Gender F/M	Total	Age, yr, mean ± SD	Med. sur. mo	95% CI, mo
MD Anderson cohort						
Superficial(Ta-Tis)	Luminal	8/26	34	65.3 ± 11.3	NA	110.7 - NA
Invasive	Luminal	11/49	60	68.3 ± 10.6	87.9	45.1 - NA
(T1 and higher)	Basal	12/22	34	67.9 ± 16.1	11	7.2 - NA
Invasive	Luminal-non p53	9/36	45	66.7 ± 11.3	91.4	41.6 - NA
(T1 and higher)	Luminal-p53	2/13	15	73.1 ± 6.2	80.8	26.3 - NA
	Basal-non p53	8/8	16	73.1 ± 8.8	10.6	6.5 - NA
	Basal-p53	4/14	18	63.2 ± 19.7	13.7	6.2 - NA
	Double negative	1/3	4	62.7 ± 8.1	NA	14.5 - NA
TCGA cohort						
Invasive	Luminal	48/164	212	68.3 ± 11.0	46.8	31.2 - 97.1
(T2 and higher)	Basal	56/123	179	68.0 ± 10.1	27.1	20.7 - 51.2
Invasive	Luminal-non p53	27/106	133	66.2 ± 11.3	NA	56.5 - NA
(T2 and higher)	Luminal-p53	21/58	79	71.9 ± 9.5	28.2	22.4 - 46.8
	Basal-non p53	29/69	98	67.6 ± 11.0	29.7	20.2 - NA
	Basal-p53	27/54	81	68.5 ± 9.0	24.1	16.8 - 104.6
	Double negative	3/14	17	65.6 ± 10.2	18.6	7.3 - NA
Lund cohort						
Superficial (Ta-pTis)	Luminal	36/80	116	69.7 ± 12.8	NA	NA - NA
Invasive	Luminal	27/112	139	70.3 ± 11.3	NA	NA - NA
(T1 and higher)	Basal	15/23	38	75.6 ± 11.1	NA	24.2 - NA
Invasive	Luminal-non p53	18/73	91	69.1 ± 11.6	NA	NA - NA
(T1 and higher)	Luminal-p53	9/39	48	72.6 ± 10.3	NA	NA - NA
	Basal-non p53	13/14	27	76.3 ± 12.1	34.8	13.4 - NA
	Basal-p53	2/9	11	73.7 ± 8.3	NA	24.2 - NA
	Double negative	2/11	13	66.8 ± 7.3	NA	NA - NA
MD Anderson paraffin-embedded formalin-fixed tissue cohort						
Invasive	Luminal	8/38	46	70.2 ± 11.6	57.3	23.4 - NA
T2 and higher)	Basal	11/18	29	69.2 ± 11.0	22.7	15.4 - NA
Invasive	Luminal-non p53	3/15	18	72.6 ± 13.7	20.4	17 - NA
(T2 and higher)	Lum-p53	5/23	28	68.7 ± 10.1	NA	36 - NA
	Basal-non p53	6/12	18	68.9 ± 11.7	37.9	12.1 - NA
	Basal-p53	5/6	11	69.7 ± 10.3	19.1	15.4 - NA
	Double Neg	3/11	14	68.0 ± 8.4	41.8	24.9 - NA

F, female; M, male; yr, year; SD, standard deviation; Med., median; sur., survival; mo, months; CI, Confidence Interval. [#]Stage is unknown in three cases. [*]Stage is unknown in two cases.

Please cite this article as: Dadhania, V., et al., Meta-Analysis of the Luminal and Basal Subtypes of Bladder Cancer and the Identification of Signature Immunohistochemical Markers ..., EBioMedicine (2016), http://dx.doi.org/10.1016/j.ebiom.2016.08.036