Human Pathology (2014) **45**, 1682–1687

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

GATA3 expression in small cell carcinoma of bladder and prostate and its potential role in determining primary tumor origin ☆

Stephania Martins Bezerra MD[a], Tamara Levin Lotan MD[a,b], Sheila Friedrich Faraj MD[a], Sarah Karram MD[a], Rajni Sharma PhD[a], Mark Schoenberg MD[b,c], Trinity J. Bivalacqua MD, PhD[b,c], George Jabboure Netto MD[a,b,c,*]

[a]*Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD 21231*
[b]*Department of Oncology, The Johns Hopkins Medical Institutions, Baltimore, MD 21231*
[c]*Department of Urology, The Johns Hopkins Medical Institutions, Baltimore, MD 21231*

Received 28 February 2014; revised 4 April 2014; accepted 9 April 2014

Keywords:
Small cell carcinoma;
Bladder;
Prostate;
Lung;
GATA3
immunohistochemistry

Summary GATA3 is a sensitive marker for urothelial carcinoma. We here evaluate, for the first time, GATA3 expression in small cell carcinoma of bladder and prostate and assess its utility in the differential diagnosis with small cell carcinoma of lung primary. Archival tissues from 60 small cell carcinomas (12 bladder, 15 lung, and 33 prostate primary cases) were used to build 2 tissue microarrays. We also assessed whole slide sections from 10 additional primary small cell carcinomas of bladder. GATA3 nuclear expression was evaluated using standard immunohistochemistry. Intensity (weak, moderate, and strong) and extent of expression were assessed in each tissue microarray spot. Extent positivity was categorized as focal (1%-25%), multifocal (>25%), and diffuse (>75%). Nuclear GATA3 expression was encountered in 7 bladder (7/22, 32%) and 2 lung (2/15, 13%) small cell carcinomas. All 33 primary prostate small cell carcinomas were negative. Among bladder tumors, strong and diffuse (>75%) GATA3 labeling was seen in 3 cases (3/22, 14%); focal positivity was observed in the 4 remaining cases (4/22, 18%). Both positive lung cases had only focal positivity. Our study is the first to reveal GATA3 expression in the small subset of lung small cell carcinoma that should be taken into consideration in assigning site of origin in advanced small cell carcinoma cases. Our novel finding of GATA3 positivity in one-third of bladder small cell carcinoma is of potential value in differentiating small cell carcinomas of prostate origin from those of bladder origin.

1. Introduction

Small cell carcinoma is an aggressive tumor that arises most commonly in the lung, accounting for up to 20% of lung cancers [1,2]. Extrapulmonary small cell carcinomas are not uncommon and arise in almost all body sites, excluding

☆ Disclosures: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article. This study was partially supported by The Brady Urological Institute–Johns Hopkins Medicine Patana Fund.

* Corresponding author. Department of Pathology, The Johns Hopkins Hospital, 401 N Broadway/Weinberg 2242, Baltimore, MD 21231.
E-mail address: gnetto1@jhmi.edu (G. J. Netto).

http://dx.doi.org/10.1016/j.humpath.2014.04.011
0046-8177/

the central nervous system. Although the urinary bladder is one of the most common primary sites, small cell carcinoma accounts for less than 1% of all primary bladder tumors [3,4]. More than 60% of the small cell carcinomas of the lung will present with metastatic disease at the time of diagnosis. Similar rates have been shown in small cell carcinomas of the bladder, in which this number can reach near 60% [5]. In a setting of metastatic small cell carcinoma of unknown origin, the identification of a primary site is critical, given the potential differences in clinical management [6-8].

GATA3-binding protein (GATA3) is a transcription factor member of the GATA family. These nuclear proteins recognize G-A-T-A nucleotide sequences in target gene promoters and activate or repress those genes [9]. It was first identified to play an important role in early T-cell development and differentiation of Th2 cells [10,11]. GATA3 function is also thought to be important in the regulation of genes such as *MUC1/EMA* involved in the luminal differentiation of breast epithelium [12,13]. Haploinsufficiency of GATA3 results in Barakat syndrome, which is characterized by hypoparathyroidism, deafness, and renal dysplasia [14].

GATA3 has been recently used as a marker for breast and urothelial differentiation. Most primary and metastatic mammary carcinomas express GATA3 (80%-90%), although its expression is reportedly lower in triple-negative tumors (67%) [15]. GATA3 has proven to be of utility in the differential of prostatic adenocarcinoma versus urothelial carcinoma [16].

In the current study, we aimed to evaluate GATA3 expression in small cell carcinoma of bladder and assess its potential role in the differential diagnosis with small cell carcinoma of lung and prostate primary.

2. Materials and methods

This study was approved by the Institutional Review Board of The Johns Hopkins Hospital.

2.1. Patient cohort

2.1.1. Tissue microarray samples

Twelve cases of small cell carcinomas of the bladder and 33 cases of small cell carcinomas of the prostate diagnosed between 1994 and 2009 were retrieved from our surgical pathology archives. All sections were reviewed by a senior uropathologist in the study (T. L.), and the diagnosis was confirmed using the 2004 World Health Organization criteria [5]. Fifteen cases of small cell carcinomas of the lung diagnosed between 2007 and 2009 were also retrieved and reviewed for the confirmation of their original diagnosis according to the 2004 World Health Organization criteria [17]. Representative formalin-fixed, paraffin-embedded archival blocks were used for the construction of 2 high-density tissue microarrays (TMAs) at the Johns Hopkins TMA Lab Core (Baltimore, MD; http://tmalab.jhmi.edu/) according to a previously described protocol [18]. Tumors and paired nonneoplastic tissue were spotted 3 to 7 times each using 1.6-mm cores.

2.1.2. Whole section samples

In addition to the 2 TMAs, our cohort also includes whole slide sections from 10 additional cases of primary small cell carcinomas of the bladder retrieved from the pathology archives in our institution from 2000 to 2013. All sections were reviewed by a senior uropathologist in the study (G. N.).

All cases included in the study, both TMAs and whole section samples, were primary tumors. In the entire cohort, electronic medical records were reviewed for pertinent clinical information including age, sex, and presence of metastasis. Importantly, clinical workup to exclude metastasis from other possible primary sites was confirmed in all cases included in the study.

2.2. Immunohistochemistry

GATA3 immunostaining was performed using a Benchmark XT automated slide stainer (Ventana Medical Systems, Inc, Tucson, AZ). Sections were deparaffinized, hydrated, and subjected to antigen retrieval with Cell Conditioning Solution (high-pH CC1 standard) for 60 minutes. Mouse monoclonal anti-GATA3 antibody (1:100 dilution, clone L50-823; Biocare Medical, Concord, CA) was applied for 44 minutes, followed by an amplification step. Slides were developed using a biotin-free, polymer detection kit (Ultra-view; Ventana Medical Systems, Inc), as per the manufacturer's instructions.

2.3. GATA3 immunohistochemical expression evaluation

Extent and intensity of GATA3 nuclear expression were assessed in each spot in the TMAs. Extent of expression was evaluated as percentage of small cell carcinoma cells showing staining. A final mean percentage of positive expression was obtained per tumor and was further categorized as focal (1%-25%), multifocal (>25%), and diffuse (>75%). Intensity of expression was evaluated in a scale (0; 1+, weak; 2+, moderate; 3+, strong). The highest spot intensity was assigned in each tumor. An identical approach was adopted in the whole slide sections in the 10 additional bladder cases. Only areas of small cell carcinoma were assessed in tumors showing other patterns of differentiation.

2.4. Statistical analysis

Findings were analyzed using STATA statistical software (version 11.2; StataCorp, College Station, TX). Expressions of GATA3 in small cell carcinomas of the bladder, prostate, and lung were compared using the χ^2 test. $P < .05$ was considered to indicate statistical significance. Performance