available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Bladder Cancer
Editorial by XXX on pp. x–y of this issue

Gene Expression Profile of the Clinically Aggressive Micropapillary Variant of Bladder Cancer

Charles Chuanhai Guo [a,†], *Vipulkumar Dadhania* [a,†], *Li Zhang* [b,†], *Tadeusz Majewski* [a], *Jolanta Bondaruk* [a], *Maciej Sykulski* [c], *Weronika Wronowska* [d], *Anna Gambin* [c], *Yan Wang* [a], *Shizhen Zhang* [a], *Enrique Fuentes-Mattei* [a], *Ashish Madhav Kamat* [e], *Colin Dinney* [e], *Arlene Siefker-Radtke* [f], *Woonyoung Choi* [e], *Keith A. Baggerly* [b], *David McConkey* [e], *John Weinstein* [b], *Bogdan Czerniak* [a,*]

[a] *Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, TX, USA;* [b] *Department of Bioinformatics and Computational Biology, University of Texas MD Anderson Cancer Center, Houston, TX, USA;* [c] *Institute of Informatics, University of Warsaw, Warsaw, Poland;* [d] *Faculty of Biology, University of Warsaw, Warsaw, Poland;* [e] *Department of Urology, University of Texas MD Anderson Cancer Center, Houston, TX, USA;* [f] *Department of Genitourinary Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA*

Article info

Article history:
Accepted February 21, 2016

Associate Editor:
James Catto

Keywords:
Micropapillary bladder cancer
Expression profile
Molecular signature
Prognosis

Abstract

Background: Progression of conventional urothelial carcinoma of the bladder to a tumor with unique microscopic features referred to as micropapillary carcinoma is coupled with aggressive clinical behavior signified by a high propensity for metastasis to regional lymph nodes and distant organs resulting in shorter survival.
Objective: To analyze the expression profile of micropapillary cancer and define its molecular features relevant to clinical behavior.
Design, setting, and participants: We retrospectively identified 43 patients with micropapillary bladder cancers and a reference set of 89 patients with conventional urothelial carcinomas and performed whole-genome expression messenger RNA profiling.
Outcome measurements and statistical analysis: The tumors were segregated into distinct groups according to hierarchical clustering analyses. They were also classified according to luminal, p53-like, and basal categories using a previously described algorithm. We applied Ingenuity Pathway Analysis software (Qiagen, Redwood City, CA, USA) and gene set enrichment analysis for pathway analyses. Cox proportional hazards models and Kaplan-Meier methods were used to assess the relationship between survival and molecular subtypes. The expression profile of micropapillary cancer was validated for selected markers by immunohistochemistry on parallel tissue microarrays.
Results and limitations: We show that the striking features of micropapillary cancer are downregulation of miR-296 and activation of chromatin-remodeling complex RUVBL1. In contrast to conventional urothelial carcinomas that based on their expression can be equally divided into luminal and basal subtypes, micropapillary cancer is almost exclusively luminal, displaying enrichment of active peroxisome proliferator-activated receptor γ and suppression of p63 target genes. As with conventional luminal urothelial carcinomas, a subset of micropapillary cancers exhibit activation of wild-type p53 downstream genes and represent the most aggressive molecular subtype of the disease

† These authors contributed equally to this study.
* Corresponding author. Department of Pathology, Unit 085, University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA. Tel. +1 713 794 1025.
E-mail address: bczernia@mdanderson.org (B. Czerniak).

http://dx.doi.org/10.1016/j.eururo.2016.02.056

with the shortest survival. The involvement of miR-296 and RUVBL1 in the development of micropapillary bladder cancer was identified by the analyses of correlative associations of genome expression profiles and requires mechanistic validation.
Conclusions: Micropapillary cancer evolves through the luminal pathway and is characterized by the activation of miR-296 and RUVBL1 target genes.
Patient summary: Our observations have important implications for prognosis and for possible future development of more effective therapies for micropapillary bladder cancer.

1. Introduction

Bladder cancer develops through two distinct tracks referred to as papillary and nonpapillary that represent different but somewhat overlapping variants of the disease with unique molecular makeups and different challenges to clinical management [1]. Superficial papillary tumors are not immediately life threatening, but they have a high tendency for recurrence. That tendency necessitates a lifetime commitment to clinical surveillance that is both invasive for the patient and costly to society [2]. Nonpapillary carcinomas have a high propensity for invasion, and at least half of them are potentially lethal due to metastatic spread [3]. Several studies have found that distinct gene expression signatures are associated with cancer progression, metastasis, and poor response to chemotherapy [4,5]. We have found that conventional urothelial carcinomas can be classified into two intrinsic luminal and basal subtypes that have distinct clinical behaviors and responses to frontline chemotherapy [6]. In addition to conventional urothelial carcinomas, a number of microscopically distinct forms of bladder cancer represent a progression of conventional disease associated with highly aggressive clinical behavior [7].

In this report, we focus on one of the most frequent variants of bladder cancer referred to as micropapillary carcinoma that exhibits unique microscopic features characterized by the presence of small infiltrating nests of tumor cells residing in empty spaces. Micropapillary bladder cancer represents, in various published series, 0.7–8% of bladder cancer and develops by a progression of the disease frequently coexisting with conventional urothelial carcinoma [8,9]. Clinically, it has a predilection for early lymph node metastases and wide metastatic spread to distant organs associated with shorter survival time compared with conventional bladder cancer of the same stage [9,10]. We report on the gene expression profile of micropapillary bladder cancer and identify unique molecular features associated with the aggressive nature of the disease that may be relevant to early detection and treatment.

2. Methods

2.1. Clinical information and tissue samples

We searched the pathology files at the University of Texas MD Anderson Cancer Center for micropapillary variants of bladder cancer identifying 43 cases, for which formalin-fixed paraffin-embedded (FFPE) tissue were available. In 35 of these cases, only the micropapillary component was analyzed; in the remaining 8 cases both conventional urothelial and micropapillary components were analyzed. Paraffin blocks from 89 randomly selected stage- and grade-matched cases of conventional urothelial carcinoma were also assembled as a reference set (Table 1 and Supplementary Table 1). Clinical data, including patient demographic characteristics, follow-up, and outcomes, were retrieved from the patients' medical records. Urothelial carcinomas were classified according to the histologic tumor grading system of the World Health Organization [11]. Levels of invasion were defined according to the TNM staging system [12]. All conventional and micropapillary carcinomas were high-grade tumors that had invaded the bladder wall and most were stage pT2 or higher. Histologic slides were reviewed to identify well-preserved tumor-rich areas of tissue with minimal amounts of stroma that contained intact pure tumor tissue (90%). Those areas were marked on the corresponding paraffin blocks. Two parallel tissue samples were taken from those areas using a 2.0-mm biopsy punch (Miltex, York, PA, USA). One of the resulting tissue cylinders was used for RNA extraction and gene expression analysis. The second was used for the construction of a tissue microarray and validation immunohistochemical analyses of selected proteins.

We also performed analyses of the micropapillary cancer expression profile on two independent publicly available cohorts of conventional urothelial carcinoma. The first set of samples represented the Cancer

Table 1 – Summary of clinical data

Tumor type	Women	Men	Total	Age, yr, mean ± SD	Median survival, mo	95% CI, mo
Con UC	22	67	89	69.6 ± 10.9	35.4	24.4–56.4
MP UC	7	36	43	70.6 ± 9.3	20.8	13.2–33.5
Con UC luminal	1	21	22	72.0 ± 14.7	49.1	22.6–164.8
Con UC p53-like	5	20	25	70.8 ± 7.8	50.1	22.7–NA
Con UC basal	16	26	42	67.5 ± 10.0	33.9	22.4–66.6
MP UC luminal	2	20	22	71.5 ± 8.8	23.6	9.9–91.5
MP UC p53-like	4	16	20	69.6 ± 10.1	18.5	8.5–33.5
MP UC basal	1	0	1	69	NA	NA

CI = confidence interval; Con UC = conventional urothelial carcinoma; NA = not applicable; SD = standard deviation; MP UC = micropapillary urothelial carcinoma.

Please cite this article in press as: Guo CC, et al. Gene Expression Profile of the Clinically Aggressive Micropapillary Variant of Bladder Cancer. Eur Urol (2016), http://dx.doi.org/10.1016/j.eururo.2016.02.056