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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, and Bogdan Czerniak^a

^aDepartment of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX

^bDepartment of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX ^cInstitute of Informatics, University of Warsaw, Warsaw, Poland

^dFaculty of Biology, University of Warsaw, Warsaw, Poland ^eDepartment of Urology, The University of Texas MD Anderson Cancer Center, Houston, TX ^fDepartment of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

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 mRNA profiling.

Outcome, measurements and statistical analysis—The tumors were segregated into distinct groups according to hierarchical clustering analyses. In addition, the tumors were

* Correspondence author. Department of Pathology, Unit 085, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030; USA Tel. 713-794-1025; bczerania@mdanderson.org (B. Czerniak).

#These authors contributed equally to this study.

C. C. Guo, V. Dadhania, T. Majewski, Y. Wang, S. Zhang performed the experiments. L. Zhang, T. Majewski, J. Bondaruk, M. Sykulski, W. Wronowska, A. Gambin, K.A. Baggerly, W. Choi, and E. Fuentes-Mattei were responsible for analysis and interpretation of data. A. M. Kamat, C. Dinney, and A. Siefker-Radtke, were responsible for the analysis of clinical data. C. C. Guo and V. Dadhania were responsible for the drafting of the manuscript. J. Weinstein, D. J. McConkey, and J. Bondaruk were responsible for critical revision of the manuscript. J. Weinstein and L. Zhang designed and supervised the statistical analysis. B. Czerniak was responsible for study concept, design, and he wrote the manuscript.

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classified according to luminal, p53-like, and basal categories using previously described algorithm. We applied IPA and GSEA 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—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 which, based on their expression, can be equally divided into luminal and basal subtypes, micropapillary cancer is almost exclusively luminal, displaying enrichment of active PPAR 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, with the shortest survival.

Conclusions—Micropapillary cancer evolves through the luminal pathway and is characterized by the activation of miR-296 and RUVBL1 target genes.

Limitations—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.

Patient summary—Our observations have important implications for prognosis and for possible future development of more effective therapies for micropapillary bladder cancer.

Keywords

micropapillary bladder cancer; expression profile; molecular signature; prognosis

Bladder cancer develops through two distinct tracks referred to as papillary and non-papillary 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]. Non-papillary 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 the 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, there are a number of microscopically distinct forms of bladder cancer that 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 which 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 co-existing 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 as compared to conventional bladder cancer of the same stage[9,10]. Here, 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.

METHODS

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 and 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 were of stage pT2 and above. Histologic slides were reviewed to identify well-preserved tumor-rich areas of tissue with minimal amounts of stroma, which 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). 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. In addition, we 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 Genome Atlas (TCGA) cohort comprised of 128 high-grade muscle invasive tumors (stage T2 and above) [13]. The second set of samples represented an independent cohort from MD Anderson Cancer Center comprised of 142 samples for which RNA was extracted from fresh frozen tumor tissue (107 men and 35 women; mean age 67.2 years $\pm$ 12.3 SD) which included 41 low-grade and 101 high-grade tumors (61 superficial, T_a–T_{1a} and 81 invasive T_{1b} and higher) [6]. For this cohort, T_{1a} and T_{1b} substaging was used to classify the tumors as superficial and invasive as previously described [14]. The use of human samples and related clinical data for this study was approved by the institutional IRB.

Microarray experiments and data processing

Total RNA from FFPE samples for microarray experiments was extracted and prepared as previously described[15]. In brief, RNA was isolated using the MasterPure Complete DNA and RNA Purification Kit (Epicenter Biotechnologies, Madison, WI, USA). RNAs (0.25–1.0 μ g) were converted to cDNAs using biotinylated and oligo-deoxythymidine primers with

Illumina reagents and were bound to streptavidin-conjugated particles. The fluorescence-labeled complementary strands were hybridized at 45°C for 18 h to Illumina HumanHT-12 DASL Expression BeadChips. After hybridization, the arrays were scanned by laser confocal microscopy using an Illumina BeadArray Reader. Array data export, processing and analysis were performed with Illumina BeadStudio v3.1.3 (Gene Expression Module V3.3.8). The selected gene signal values were transformed to logarithmic scale and normalized by the sample-wise medians. For clustering, we used average linkage with Euclidian distance as the similarity metric. To select gene sets associated with micropapillary bladder cancer, we used the combination analysis with t-test p-values and mean log2-fold differences. P-values <0.05 and mean log2-fold differences >0.5 were used as the criteria for selecting significant genes. We computed the false discovery rate using cutoffs of $p < 0.05$ and fold change > 1.4 as being equivalent to $FDR < 0.05$. Pathway analyses were performed using Ingenuity Pathway Analysis (IPA) software (Ingenuity® Systems, CA). Significance of altered pathways (p-values) was calculated according to one-sided Fisher's exact test. In addition two other measurements were presented to assess the genes showing overexpression or downregulation: "Z-score" and "ratio". A Z-score indicates a relationship to the mean in a group of scores. A Z-score can be positive or negative, specifying whether the pathway is overexpressed or downregulated. The Z-scores were calculated according to A. Kramer et al[16]. The ratio values used for the canonical pathways analyses were calculated as the number of molecules with the expression levels above or below the mean divided by the total number of molecules in that pathway. For calculation of p-values and Z-scores for miR-296 regulated genes, we used the predicted targets of miR-296 based on the seed RNA sequence. In addition, the gene set enrichment analysis (GSEA) method was used to evaluate the significance of pathway changes. Both the conventional urothelial and the micropapillary bladder carcinoma samples were classified initially into luminal and basal subsets using the previously described algorithm[6]. A subtype of luminal cancers characterized by upregulation of p53 downstream regulatory genes was also identified[6].

Micro RNA profiling

The expression levels of miR-296 were determined from our unpublished data on micro RNA profiling performed on a subset of 20 micropapillary tumor samples and 20 conventional urothelial carcinomas from the same cohort of bladder cancers (Supplementary Table 1). The analyses were completed using TaqMan Low Density Array (TLDA) with the TaqMan Array Human MiRNA Card A v2.0 according to manufacturer's instructions[17].

Tissue microarrays and immunohistochemistry

The expression levels of selected genes were tested on parallel tissue microarrays (TMA) comprising the same cohort of 89 conventional and 43 micropapillary FFPE bladder tumor samples from which cDNA microarray was prepared. The tissue microarrays were designed and prepared as previously described[18]. In brief, tissue microarrays were constructed with a tissue arrayer (Beecher Instruments, Silver Spring, MD). Immunohistochemical staining was performed with mouse monoclonal antibodies against human CD44 (H-CAM DF1485 clone, 1:75 dilution; Leica Biosystems, Buffalo Grove, IL), KRT14 (LL002 clone, 1:50 dilution; BioGenex, Fremont, CA), GATA3 (HG3-31 clone, 1:100 dilution; Santa Cruz

Biotechnology Inc., Santa Cruz, CA) and human uroplakin 2 (BC21 clone, 1:100 dilution; Biocare Medical, Concord, CA). Immunohistochemical stains were performed using the Bond-Max Autostainer (Leica Biosystems, Buffalo Grove, IL). The slides were incubated with the primary antibodies, followed by the visualization reagent linked to a dextran polymer backbone with DAB (3, 3-diaminobenzidine) as a chromogen solution. The slides were counterstained with Mayer's hematoxylin. Staining was scored manually by two pathologists.

Statistical Analyses

We used R packages in Bioconductor (<http://www.Bioconductor.org>) to process the data and carry out the statistical analyses. Cox proportional hazards models and Kaplan-Meier methods were used to assess the relationship between the overall survival and molecular subtypes. The response to chemotherapy in molecular subtypes of micropapillary cancer (luminal versus p53-like) for primary bladder cancer was evaluated by downstaging to pT0N0 at cystectomy and for metastatic disease using RECIST criteria on CT imaging. The significance in chemotherapy response rates was assessed by the chi-squared and Fisher tests.

RESULTS

We performed whole genome mRNA expression profiling and unsupervised hierarchical clustering analysis on a cohort of 43 micropapillary bladder cancers and a reference set of 89 conventional urothelial carcinomas (Table 1 and Supplementary Table 1). The micropapillary cases included 35 for which only mRNA from the micropapillary component was available and eight for which mRNA was extracted from both conventional urothelial carcinoma and micropapillary components of the same tumors.

Over 6000 genes were differentially expressed in micropapillary carcinomas as compared with conventional carcinomas (Fig. 1A and Supplementary Table 2). To define the molecular mechanisms involved in the development of micropapillary carcinoma, we initially used the "canonical pathways" function in Ingenuity Pathway Analysis (IPA, Ingenuity Systems; <http://www.ingenuity.com>) on the gene expression signature of micropapillary cancers. We found that micropapillary tumors were enriched with expression signatures involved in multiple important oncogenic pathways converging on transformation (mechanisms of cancer, mechanisms of glioma/glioblastoma, RhoA, and p53), cell cycle regulation (cyclins, G1/S checkpoint), DNA damage repair (BRCA1), and signal transduction (ephrin signaling) (Fig. 1D and Supplementary Table 3). Surprisingly, a micropapillary expression signature was present in the conventional component of the tumors which contained foci of micropapillary carcinoma. We performed multiple clustering analyses using all differentially expressed genes of micropapillary tumors as well as similar analyses using 1000, 100, and 20 top upregulated and down regulated genes. When analyzed by hierarchical clustering, the tumors segregated into two distinct clusters referred to here as clusters A and B (Fig. 1, B and C). Cluster A contained almost exclusively conventional urothelial carcinomas whereas cluster B contained most of the micropapillary tumors. Interestingly, almost all samples of pairs of conventional and micropapillary carcinomas from the same tumors co-segregated

with micropapillary carcinomas in cluster B. The analyses indicated that the micropapillary expression signature was present in conventional carcinomas that contained foci with micropapillary features. Moreover, a small fraction (5.6%; 5/89) of conventional carcinomas segregated into cluster B together with the micropapillary type. The survival analyses using the microscopically identified micropapillary features as well as using the expression signature with hierarchical clustering showed that micropapillary tumors were associated with more aggressive clinical behavior when compared with conventional urothelial carcinoma (Fig. 1E and F).

Recent molecular studies, including our own observations, have divided bladder cancer into two molecular subtypes that contain basal and luminal gene expression patterns recapitulating the expression features of normal urothelium[6, 13,19]. We analyzed these gene expression signatures in micropapillary cancer and asked whether the intrinsic molecular types of conventional urothelial carcinoma of the bladder applied to its micropapillary variant. We used a previously developed algorithm that includes the markers of luminal, p53-like, and basal types (Fig. 2A)[6]. The reference set of conventional urothelial carcinoma was separated into two major groups. The first group (52%; 47/89) was characterized by high mRNA expression levels of luminal markers such as KRT20, GATA3, uroplakins, ERBB2, ERBB3, CD24, FOXA1, and XBP1, among others referred to as luminal subtype. A subset of luminal type specimens (28%; 25/89) was distinguished by an activated wild-type p53 gene expression signature and was referred to as p53-like. The remaining conventional urothelial carcinomas (47%; 42/89) were characterized by high expression levels of basal markers such as CD44, CDH3, KRT5, KRT6, and KRT14 and were referred to as basal subtype. In contrast, the majority (98%; 42/43) of micropapillary cancer was of luminal type (Fig. 2B) with only one case showing the basal gene expression signature. Similarly, the two components of the micropapillary tumors for which pairs of conventional and micropapillary variants of the same tumor were analyzed showed the expression signature of luminal subtype. In survival analysis, the p53-like subset of micropapillary carcinomas appeared to be the most aggressive among the molecular subtypes (Fig. 2C). We next verified the expression patterns of signature luminal and basal markers by immunohistochemistry using tissue microarrays containing the same cases as were analyzed for the gene expression profiles (Fig. 2D). Micropapillary cancers were consistently positive for expressions of markers of terminal luminal differentiation such as GATA3 and uroplakin 2. In contrast, they were consistently negative for basal markers such as CD44 and KRT14.

With the information of molecular subtypes in micropapillary carcinoma we revisited the clustering information from Fig. 1B and analyzed individual cases which co-clustered with a different subtype i.e. a few cases classified as conventional carcinoma that co-clustered with micropapillary cancer in cluster B and one case of micropapillary tumors that co-clustered with conventional carcinomas. The only case of micropapillary carcinoma which co-clustered with conventional carcinoma in cluster A was of basal type which microscopically exhibited squamous differentiation in the conventional component. In addition, one of the paired samples corresponding to a conventional component of the micropapillary tumors co-segregated with conventional tumors in cluster A. All five cases of conventional tumors

which co-clustered with micropapillary tumors did not exhibit any divergent differentiation and were of luminal type, one of which was of the p53-like subtype.

In order to assess the relationship between the sensitivity to chemotherapy and the molecular profile of micropapillary cancer, we evaluated the responses to frontline chemotherapy of primary tumors in cystectomy specimens by downstaging to pT0N0 and for metastatic disease on CT imaging by RECIST criteria. The data were available for 6 out of 22 patients in the luminal group and for 11 of 20 in the p53-like group. The response rate was 4/6 (66%) in the luminal group and 5/11 (45%) in the p53-like group. The data suggest a higher response rate in the luminal category but the difference is statistically not significant ($p = 0.74$).

Using the “upstream regulator” function of IPA, we confirmed that, as with conventional luminal cancers, micropapillary cancers were enriched with PPAR γ target genes and showed downregulation of the p63 signature genes that are enriched in basal bladder cancers (Fig. 3 and Supplementary Tables 4–6). However, the most striking feature of the micropapillary samples was an overexpression of nearly 300 miR-296 target genes (Fig. 3A and Supplementary Table 7). Since our data indicates that micropapillary bladder cancer develops almost exclusively from luminal conventional urothelial carcinoma, in order to identify the mechanisms that may drive its development, we performed additional analyses comparing the expression profiles of the micropapillary specimens with luminal conventional urothelial carcinomas (Supplementary Fig. 1 and Supplementary Table 8). The canonical pathway analysis identified a similar set of enrichments involving mechanisms of cancer, cell cycle and DNA damage (Fig. 4A and Supplementary Table 9). The analyses of upstream regulators confirmed upregulation of miR-296 target genes, and the microRNA profiling confirmed downregulation of miR-296 in micropapillary cancer (Fig. 4B–4D and Supplementary Table 10). RUVBL1 was overexpressed and in both the upstream regulator and gene set enrichment analyses and its downstream pathway was upregulated in the micropapillary tumors when compared to the conventional urothelial carcinomas (Fig. 4B, E, and F). The upregulation of RUVBL1 downstream targets included such genes as KDM4B, IGFBP3 and ADAM15, which are involved in cell growth, DNA damage repair and metastasis (Supplementary Fig. 2, Supplementary Table 11)[20–22].

Finally, we addressed the issue of whether the micropapillary expression signature can be identified in the independent cohorts of conventional bladder cancer and how it relates to other molecular classifications of bladder cancer. First, we analyzed the publically available TCGA cohort of conventional bladder cancer and searched whether the micropapillary signature can be identified in these tumors. The bladder tumor samples were first classified into luminal and basal phenotypes and analyzed for the expression pattern of top 50 upregulated and top 50 downregulated gene characteristic of micropapillary cancer. As expected, this approach has identified a small subset (**3.9%**) of conventional bladder cancers expressing a micropapillary signature all of which were in the luminal group. In order to assess the relationship of the micropapillary signature to other molecular classifications of bladder cancer we used an independent MD Anderson cohort of fresh bladder tumor samples comprised of 142 conventional bladder cancers and applied the G. Sjødhal et al. algorithm [23]. Similar to the TCGA cohort, this approach identified a small proportion of cases with a

micropapillary signature within a genomically unstable group which overlaps with the luminal and p53-like categories in our classification.

DISCUSSION

We conclude that micropapillary bladder cancer is characterized by widespread dysregulation of its expression profile, affecting approximately 30% of the protein-coding genome. The expression signature is already present in the conventional component of urothelial carcinomas that show progression to the micropapillary variant. The micropapillary expression signature is also present in a small fraction of bladder cancers that microscopically show features of conventional urothelial carcinoma. The change in expression pattern affects multiple oncogenic pathways focused on transformation, cell cycle regulation, DNA damage repair and signal transduction. We also found that micropapillary cancers are almost exclusively of luminal type. The precursor conventional urothelial carcinomas that progressed to the micropapillary variant consistently exhibited the same luminal signature. In addition, they showed the expression signature of altered transcription factors that was similar to the conventional luminal variant of bladder cancer. A subset of luminal micropapillary bladder cancers defined as p53-like represented the most aggressive variant of the disease. The so-called p53-ness has been associated with chemo-resistance to cisplatin-based neoadjuvant chemotherapy[6, 24]. It is important to mention that the p53 gene signature and chemo-resistance were not related to p53 mutations. Both *de novo* and induced chemo-resistance in conventional carcinomas were associated with wild-type p53 expression signatures[6, 24]. Moreover, in a recent study, we showed that tumors that develop resistance to chemotherapy exhibit phenotypic plasticity with switch to p53-ness[24]. In fact, the analysis of therapeutic responses in molecular subtypes of micropapillary cancer suggests that the p53 variant of micropapillary cancer is more resistant to chemotherapy, however the difference was not statistically significant.

The molecular subtypes of bladder cancer are reminiscent of those originally identified in human breast cancers which can also be divided into luminal and basal subtypes using a similar set of markers[6, 19]. Progression of carcinomas to tumors with micropapillary features also occurs in a fraction of cancers of multiple sites, including breast carcinomas[25,26]. Expression profiling of micropapillary breast cancer reveals similarities to micropapillary cancers of the bladder that they are of luminal subtype[26,27]. Three other groups used genome expression profiling to sub-classify bladder cancer into distinct subtypes – one group concluded that there were two [19]subtypes, another that there were three[6,28,29] and the third that there were four[13]. Although these classifications used different names for their respective classes, all of them used a similar set of markers that is surprisingly similar to those identified in breast cancers[6,13,19,28,29]. Specifically these markers recapitulate the differentiation pattern of normal urothelium and can be divided into two main groups reflecting an expression pattern of basal and intermediate/luminal urothelial cell layers[28,29]. Moreover, the subtypes identified by the three groups show striking similarities with at least three of the previously identified subtypes (squamous, genomically unstable, and infiltrated)[23,30]. In addition, we were able to identify the expression signature of micropapillary cancer in a small fraction of conventional carcinomas in our study set and in two independent cohorts (TCGA and MD Anderson). In both

instances this signature was identified in subsets of tumors exhibiting luminal expression phenotype.

Most importantly, our analyses show that downregulation of miR-296 with upregulation of its target genes and activation of the RUVBL1 pathway appear to drive the expression signature of micropapillary cancer and contribute to its development. Down-regulation of miR-296 has been reported in many human cancers[31–34]. It typically occurs in later phases of carcinogenesis and is associated with the progression to aggressive disease[33,34]. It acts as a global repressor of tumorigenicity, and the loss of function upregulates multiple oncogenic pathways involved in tumor progression, including those controlled by Scrib, HMGA1 and Pin1[31,32,34]. Studies of miRNAs in bladder cancer indicate that their specific species can be associated with bladder cancer behavior and chemosensitivity. [35] Specifically miRNA-296-5p modulation was shown to be associated with altered viability of cell lines exposed to cisplatin. [35] Similarly, activation of RUVBL1 has been reported in many cancers and is typically associated with clinically aggressive forms of disease[36,37]. The RUVBL1 molecule belongs to the family of AAA+ ATPases that act as scaffolding proteins of various chromatin-remodeling complexes and control diverse cellular functions such as transcription, DNA damage repair, proliferation and invasion[36,37]. They are implicated in cellular transformation by interacting with Myc, β -catenin and p53. In summary, our study shows that miR-296 and RUVBL1 play important roles in the development of micropapillary bladder cancer and may represent attractive new diagnostic, prognostic and therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Whole genome mRNA expression profiling of micropapillary and conventional bladder cancer

(A) The top 50 upregulated and top 50 downregulated genes in 43 cases of micropapillary cancer compared to 89 cases of conventional urothelial carcinoma. (B) Hierarchical cluster analysis of the cohort shown in A using the top 10 upregulated and the top 10 downregulated genes identified in micropapillary cancer. (C) The distribution of samples in clusters A and B identified by hierarchical clustering analysis. (D) Expression of the top 10 canonical pathways enriched in micropapillary cancers compared with expression in conventional bladder cancers. (E) Kaplan-Meier analysis of survival in micropapillary and conventional bladder cancers. (F) Kaplan-Meier analysis of survival in clusters A and B. P, one-tailed Fisher's exact p-value; r, ratio of expression values. Con UC, conventional urothelial carcinoma; MP UC, micropapillary urothelial carcinoma.

Figure 2. Luminal and basal molecular subtypes in conventional and micropapillary bladder cancers

(A) The expression of luminal, p53, and basal markers in molecular subtypes of conventional and micropapillary bladder cancers. (B) The distribution of molecular subtypes in conventional and micropapillary bladder cancers. (C) Kaplan-Meier plots of molecular subtypes of conventional and micropapillary bladder cancers. (D) The immunohistochemical expression of signature luminal and basal markers in representative luminal and basal cases of conventional bladder cancer as well as representative luminal micropapillary cancer.

Figure 3. Expression pattern of signature transcriptional regulators in conventional and micropapillary bladder cancers
(A) The top 10 upstream regulators enriched in micropapillary cancer. (B) Expression patterns of PPAR γ and p63 target genes in molecular subtypes of conventional and micropapillary bladder cancer. (C) PPAR γ expression signatures of micropapillary and conventional basal bladder cancers compared by GSEA. (D) p63 expression signatures of micropapillary and conventional basal of bladder cancers compared by GSEA. P, one-tailed Fisher's exact p-value; r, ratio of expression values.

Figure 4. Enrichment of canonical pathways and upstream regulators in micropapillary cancers as compared with conventional luminal urothelial carcinomas

(A) The top 10 canonical pathways enriched in micropapillary cancer. (B) The top 10 upstream regulators enriched in micropapillary cancer. (C) Expression pattern of miR-296 and RUVBL1 target genes in molecular subtypes of conventional and micropapillary subtypes of bladder cancer. (D) Expression levels of miR-296 in conventional and micropapillary bladder cancers. (E) GSEA of RUVBL1 expression signature by comparing micropapillary with conventional luminal subtype of bladder cancer. (F) Expression levels of RUVBL1 in conventional and micropapillary bladder cancers. P, one-tailed Fisher's exact p-value; r, ratio of expression values.

Table 1

Summary of Clinical Data

Tumor Type	Female	Male	Total	Age (year) (mean $\pm$ stdev)	Median Survival (month)	95% Confidence Interval (month)
Con UC	22	67	89	69.6 $\pm$ 10.9	35.4	24.4 – 56.4
MP UC	7	36	43	70.6 $\pm$ 9.3	20.8	13.2 – 33.5
Con UC luminal	1	21	22	72.0 $\pm$ 14.7	49.1	22.6 – 164.8
Con UC p53-like	5	20	25	70.8 $\pm$ 7.8	50.1	22.7 – NA
Con UC basal	16	26	42	67.5 $\pm$ 10.0	33.9	22.4 – 66.6
MP UC luminal	2	20	22	71.5 $\pm$ 8.8	23.6	9.9 – 91.5
MP UC p53-like	4	16	20	69.6 $\pm$ 10.1	18.5	8.5 – 33.5
MP UC basal	1	0	1	69	NA	NA