Expression of Epithelial-Mesenchymal Transition Related Markers in Plasmacytoid Urothelial Carcinoma of the Urinary Bladder

CURRENT STATUS: UNDER REVIEW

BMC Urology • BMC Series

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DOI:
10.21203/rs.2.21996/v1

SUBJECT AREAS
Urology & Nephrology

KEYWORDS
bladder, urinary bladder, plasmacytoid, urothelial carcinoma, E-cadherin, Snail, epithelial-mesenchymal transition
Abstract

Background

Plasmacytoid urothelial carcinoma (PUC) of the urinary bladder is a variant of urothelial carcinoma that carries a poor prognosis. The epithelial-mesenchymal transition (EMT) has been demonstrated to contribute to tumor progression. As the cause of the increased aggressiveness of PUC is unknown, we investigated PUC and EMT-related marker expression. The proportion of plasmacytoid variant histology

Materials and Methods

A total of 633 bladder carcinoma cases diagnosed from 2006 to 2014 at the Nippon Medical School Hospital were analyzed. Twelve patients were found to have plasmacytoid histology and diagnosed with PUC. Slides were evaluated for percentage of plasmacytoid variant, and stained for E-cadherin, N-cadherin, Vimentin, Fibronectin and Snail expression.

Results

The incidence of PUC was 1.9% (12/633). The median patient age at diagnosis was 71 years (range, 60–80 years) and the male-female ratio was 11:1. All but three patients had stage T2b or higher. The median overall survival was 10 months. In 10/12 cases, Snail and N-cadherin were positive. Vimentin was positive in 9/12 cases. Fibronectin was positive in 8/12 cases. While E-cadherin was negative in 10/12 cases. Nine cases showed >10% plasmacytoid component. Eight of the nine patients (88.9%) with >10% plasmacytoid component died.

Conclusions

The results indicate that PUC may induce EMT and may be associated with high invasion.

Background
Plasmacytoid urothelial carcinoma (PUC) of the urinary bladder was first reported in 1991 as a similar histological type to plasma cell. [1] The World Health Organization (WHO) working group classified PUC as a variant of urothelial carcinoma (UC). These tumor cells have eosinophilic cytoplasm and eccentrically placed, enlarged hyperchromatic nuclei with small nucleoli. [2] The prognosis of PUC of the bladder is worse than that of conventional UC. [3] However, why PUC is more aggressive is unknown.

Recent research suggests that the epithelial-mesenchymal transition (EMT) is an important factor related to tumor progression and metastasis. [4] [5] EMT is a process initially observed in embryonic development in which cells lose epithelial characteristics and gain mesenchymal properties to increase motility and invasion. [4] Furthermore, a recent study reported that Snail is a key regulator of EMT. [6] Snail is a super family of zinc-finger transcription factors that was first identified in Drosophila melanogaster. [7] Snail induces EMT, in part, by directly repressing epithelial markers such as E-cadherin and by upregulating mesenchymal markers such as N-cadherin, Vimentin and Fibronectin. Immunohistochemistry has shown downregulated or negative E-cadherin expression in the majority of PUC. [3] [8] Thus, PUC may induce EMT. Therefore, EMT may be associated with PUC progression. The present study examined the expression status of EMT-related markers (E-cadherin, N-cadherin, Vimentin, Fibronectin and Snail) in PUC.

Whether survival is related to the proportion of plasmacytoid variant histology is unknown. Thus, we assessed the association between the proportion of plasmacytoid variant histology and survival in PUC patients. Furthermore, we also report clinical outcome information.

Material And Methods

Patients and Samples
The cohort under investigation comprised 12 patients who had bladder carcinoma with plasmacytoid histology at our institution between March 2006 and August 2015. All hematoxylin and eosin stained glass slides were retrieved and reviewed to confirm the diagnosis using the WHO definition of “plasmacytoid variants”. Having been compiled for research purposes, this group represents patients for whom pretreatment archival paraffin-embedded tissue blocks and data from complete clinical follow-up were available. Tumors were graded histologically in accordance with WHO classifications and were staged as per the TNM staging system of the Union for International Cancer Control (2009). The amount of PUC as a percentage was evaluated in the transurethral resection of bladder tumor (TURBT) and compared with that in the cystectomy when available.

Immunohistochemistry

Immunostaining was performed on at least one representative paraffin section using routine laboratory standard protocols. The antibodies used on paraffin-embedded tissues included EMA (Dako, Glostrup, Denmark), CK7 (Dako), CK20 (Dako), E-cadherin (Nichirei, Tokyo, Japan), N-cadherin (TaKaRa, Otsu, Japan), Vimentin (Dako), Fibronectin (Abcam, Cambridge, UK) and Snail (Abcam). The stained tumor tissues were evaluated blindly with respect to clinical patient data. Staining was assessed using a semiquantitative scoring system (0, 1+, 2+, and 3+). Immunohistochemical staining was evaluated as follows: 0, no staining of tumor cells; 1+, faint staining in less than 10% of tumor cells; 2+, weak or moderate staining in more than 10% of tumor cells; and 3+, strong staining in more than 10% of tumor cells. Staining intensity of 0 or 1+ was considered negative, while 2+ or 3+ staining was considered positive. Negative controls were incubated without the primary antibody.

Results
Clinical data

The clinicopathologic features of the 12 patients are shown in Table 1. The median age of patients was 71 years (range, 60–80 years) and there was only one female patient. TNM stage was cT1N0 in three patients, cT2bN0 in three, cT3b-4aN0 in four, cT2bN1 in one, and cT3bN2 in one. The initial diagnosis of plasmacytoid carcinoma of the bladder was made on TURBT in 11 cases and cystoprostatectomy in one case. None of the 12 cases had a prior history of bladder cancer. Eleven patients had follow-up information available, while one was lost to follow-up. Eight of the patients died of their disease from 3 to 15 months (median 9 months), while three patients were alive from 29 to 36 months (median 36 months). One patient death was attributed to chemotherapy. With a median follow-up of 9.5 months, the overall median survival was 10 months. The 1-year survival rate was 33.3% for all patients.

Cases were stratified based on the percentage of plasmacytoid variant histology. Nine cases (75%) showed >10% plasmacytoid component, while three cases (25%) showed <10% plasmacytoid component. Eight of the nine (88.9%) patients with >10% plasmacytoid component died. The 1-year survival rate was 11.1% for >50% plasmacytoid patients. Conversely, the 1-year survival rate was 100% for <10% plasmacytoid patients.

Immunohistochemistry

The immunohistochemical findings in the 12 cases are summarized in Table 2. EMA was positive in 5/12 cases (41.7%), CK7 was positive in 7/12 cases (58.3%), CK20 was positive in only 2/12 cases (16.7%). E-cadherin was negative in 10/12 cases (83.3%). N-cadherin and Snail were positive in 10/12 cases (83.3%). Vimentin was positive in 9/12 cases (75%). Fibronectin was positive in 8/12 cases (66.7%). Snail was localized in the nucleus of PUC cells. E-cadherin and N-cadherin were localized in the cytoplasm of PUC cells. Typical UC
was positive for E-cadherin. Representative cases of immunohistochemical staining are presented in Fig. 1.

Discussion

PUC is recognized as a rare and aggressive variant of UC, which often presents at a high stage and carries a poor prognosis. [3] Sahin et al. first described this tumor in 1991. [1] Mai et al. reported an incidence of 2.7% of PUC in a series of muscle invasive UC. [9] In this study, 9 of 12 tumors were invasive. Two-thirds of our PUC patients died during follow-up. The median age at diagnosis was 71 years, a finding similar to other recent reports. We recognized that the poor prognoses of these cases are due to the high invasion and the high clinical stage at presentation. [3] However, it is unclear how often PUC shows high invasion.

EMT is an important step during epithelial tumor metastasis. [10] EMT causes changes in cell-cell and cell-extracellular matrix interactions resulting in transmigration of cancer cells, thus leading to metastasis. [11] [12] E-cadherin is a cell-cell junction protein that is frequently downregulated or lost during EMT, whereas expression of N-cadherin, Vimentin, Fibronectin and Snail are acquired during this process. In this study, E-cadherin expression was largely negative, while N-cadherin, Vimentin, Fibronectin and Snail were mostly positive. The present study reported a possibility of EMT in PUC.

E-cadherin is a key structure protein in maintaining both the stability of adhesion between epithelial cells and the stability of tissues. [13] Recent studies have demonstrated that loss of E-cadherin expression may correlate with high grade and advanced stage of UC. [14] [15] Other studies reported that the majority of PUC cases show low E-cadherin expression. [3, 8] Keck et al. reported that most PUC with loss of membranous E-cadherin show a nuclear accumulation of E-cadherin. E-cadherin also serves as an independent prognostic factor for reduced overall survival of patients with muscle-invasive bladder
cancer who were treated with radical cystectomy and adjuvant chemotherapy.[16] In this study, 10 of 12 cases (83.3%) showed largely negative membranous E-cadherin.

Snail is considered a key regulator of EMT and, therefore, of tumor progression. Bruyere et al. reported that Snail expression predicts tumor recurrence in superficial bladder cancer. [17] Kosaka et al. reported that Snail expression is a prognostic predictor of disease-free survival and disease-specific survival in upper urinary tract UC. [18] We also previously reported that Snail expression may predict poor outcome in bladder cancer patients treated with neoadjuvant chemotherapy. [19] In this study, Snail was mostly positive in 83.3%. PUC also may predict poor outcome by Snail. Further studies with a large cohort of PUC patients are needed to confirm this result.

Whether survival is related to the proportion of plasmyctoid variant histology has been unknown. Here we assessed the association between the proportion of plasmyctoid variant histology and survival in PUC patients. Analysis of the correlation between amount of PUC and outcome revealed that the three patients with < 10% plasmyctoid component did not die from cancer, while 88.9% of those with > 10% plasmyctoid component died from disease. This result may demonstrates the importance in identifying the amount of PUC.

Conclusions

PUC may induce EMT and may be associated with high invasion. However, we have not shown a direct association with EMT in PUC in the present study. More detailed studies are needed to address this question.

Abbreviations

PUC:Plasmyctoid urothelial carcinoma; WHO:World Health Organization; UC:Urothelial carcinoma; EMT:Epithelial-mesenchymal transition; TURBT:Transurethral resection of
bladder tumor

Declarations

Acknowledgments

Kyoko Wakamatsu provided technical support.

Author contributions

SN evaluated immunohistochemical staining, performed the statistical analyses, and drafted the manuscript. YS assisted with the statistical analysis and helped draft the manuscript. JA and YE supervised the research and helped write the manuscript. AS evaluated the immunohistochemistry and helped draft the manuscript. TH revised the manuscript. GK did the histological review of the samples and reviewed the manuscript. YK participated in the study conception and design, data analysis, interpretation, drafting, and final approval of the manuscript. All authors have read and approved the final manuscript.

Funding

No funding was obtained for this study.

Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. Approval of the protocol was obtained from the institutional review board at Nippon Medical School.

Consent for publication

All of the details can be published and consent for publication was not required for this study.
Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, Tables 1-2 are provided in the Supplementary Files section.

Figures
Figure 1

A. Hematoxylin and eosin staining: the tumor cells have eosinophilic cytoplasm and eccentrically placed, enlarged hyperchromatic nuclei with small nucleoli. B. Snail-positive tumor cells. C. N-cadherin-positive tumor cells. D. E-cadherin-negative tumor cells of PUC with an E-cadherin-positive typical urothelial carcinoma.

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Tables.docx