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ANATOMICAL PATHOLOGY

High grade neuroendocrine carcinoma of the urinary bladder treated by radical cystectomy: a series of small cell, mixed neuroendocrine and large cell neuroendocrine carcinoma

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Summary

High grade neuroendocrine carcinomas (HGNEC) treated by cystectomy often carry an original diagnosis of typical urothelial carcinoma (UC). The correct diagnosis of HGNEC is critical in influencing the decision for early chemotherapy, potentially followed by cystectomy. The objective of this study was to characterise the features of HGNEC treated by radical cystectomy. The study consisted of 79 patients with HGNEC including small cell (68 patients), large cell neuroendocrine (LCNEC) (5 patients) and mixed neuroendocrine (mixed-NEC) carcinoma (6 patients) matched with 122 patients with UC, treated at our institution between 1987 and 2014. Morphometric analysis for cell and nuclear size as well as immunophenotyping for neuroendocrine markers and cell-cycle regulators were applied to tissue microarrays. Small cell, LCNEC and mixed-NEC are a morphological spectrum of high grade neuroendocrine carcinoma with overlapping histological features, identical immunophenotype, Ki-67 proliferative rate and patient outcomes. Finally, the nuclear size criteria is misleading as HGNEC, particularly cases of LCNEC and mixed-NEC, may have enlarged nuclei compared to small cell carcinomas and are more prone to be misdiagnosed as UC, thereby preventing appropriate management.

Key words: Cystectomy, high grade neuroendocrine carcinoma, large cell neuroendocrine carcinoma, small cell carcinoma, urinary bladder, urothelial carcinoma.

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INTRODUCTION

The diagnosis of small cell carcinoma of the urinary bladder holds significant clinical implications. Patients with small cell carcinoma are optimally treated with initial systemic chemotherapy, followed by re-evaluation and consideration for definitive local therapy with either cystectomy or radiation therapy. Indeed, recent studies show that outcomes with small cell carcinoma are significantly impacted by receipt of neoadjuvant chemotherapy, with 5-year cancer specific survival rates improving from 38% to 78% in patients that received neoadjuvant chemotherapy in one study.[1] The chemosensitive nature of small cell carcinoma has been validated in reports from multiple institutions that have recommended neoadjuvant chemotherapy for advanced (Stage III and IV) disease as well as the utilisation of multimodal treatment approaches in addition to cystectomy.[2–4] The consensus opinion based on more recent studies is that the maximal therapeutic benefit is obtained when patients receive platinum based chemotherapy in a neoadjuvant setting prior to cystectomy.[5,6] For this to be a feasible therapeutic strategy, it requires an accurate diagnosis.

The spectrum of high grade neuroendocrine carcinomas (HGNEC) of the urinary bladder encompasses conventional small cell carcinomas and the rarer entity of large cell neuroendocrine carcinomas (LCNEC). Small cell carcinoma exhibits diffuse sheets of uniform, hyperchromatic cells with scant cytoplasm, nuclear molding, granular chromatin, inconspicuous nucleoli and other features include brisk mitotic activity, variable necrosis, the presence of crush artifact and the Azzopardi phenomenon.[7,8] At present, the World Health Organization (WHO) recommends diagnosing small cell carcinoma on 'morphological grounds alone' even in the absence of evidence of neuroendocrine differentiation and irrespective of the presence of other histopathological variants.[8] The first reports of LCNEC of the bladder were based on the Armed Forces Institutes of Pathology (AFIP) criteria that were used to diagnose its counterpart in the lung.[9–11] By the authors' own admission, the 'chief distinguishing features' between small cell and LCNEC was the 'size of the tumour cells (smaller in the former) and the presence of prominent nucleoli and a lower nuclear-to-cytoplasmic ratio in the latter'.[10] Current morphological criteria used to diagnose LCNEC of the bladder mirror WHO recommendations for its diagnosis in the lung, which includes architectural features such as organoid nesting, trabecular growth, rosettes, perilobular palisading, with the tumour being comprised of large cells with moderate to abundant cytoplasm, prominent nucleoli and brisk mitotic activity.[7,12–14] Importantly, confirmatory diagnosis of pulmonary LCNEC based on the WHO criteria requires the use of ancillary tools such as expression of markers of neuroendocrine differentiation or the presence of characteristic neurosecretory granules on ultra-structural studies.[13,14] However, at present, no definitive consensus diagnostic criteria exist for the diagnosis of urinary bladder LCNEC.

Herein, we carried out a histopathological characterisation of 79 cases of HGNEC which included 68 cases of conventional small cell carcinoma, five cases of LCNEC and six mixed neuroendocrine carcinomas (mixed-NEC) which had features of both small cell and LCNEC, matched with 122 cases of

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urothelial carcinoma (UC). This study includes a comparison of histopathological attributes which define conventional small cell carcinoma and LCNEC as well as outcomes. The motivation for conducting this study was driven by the need for refining diagnostic criteria, so as to accurately diagnose HGNEC and ensure appropriate patient management.

MATERIALS AND METHODS

Patient specimens

Permission for this study was obtained from The Mayo Clinic Institutional Review Board. The study cohort consisted of 79 patients identified with HGNEC, treated with radical cystectomy at our institution between 1987 and 2014. While following the conventional diagnostic criteria for HGNEC, particular attention was paid to nuclear chromatin pattern that was dispersed and granular regardless of nuclear size, and cases were identified during pathological re-review of all patients that underwent radical cystectomy between 1987 and 2014. A total of 1401 radical cystectomy cases with invasive (pT1 or greater) bladder cancer were re-reviewed by one of the authors (JCC) to identify 79 cases of HGNEC. Verification of histopathological diagnosis and subclassification of HGNEC into small cell, mixed-NEC and LCNEC was performed by two of the authors (SG and JCC). These patients were then matched for age, gender, body mass index (BMI), Eastern Cooperative Oncology Group (ECOG) status, bladder cancer stage (as per the 2010 AJCC staging criteria) and year of surgery with 122 patients with UC treated by radical cystectomy (Table 1). Haematoxylin and eosin (H&E) stained slides for morphological assessment, and formalin fixed, paraffin embedded tissue specimens for downstream analysis (tissue microarray generation and immunohistochemistry), were retrieved from the Mayo Clinic surgical pathology archives. Patient follow-up included analysis of bladder cancer specific survival.

Immunohistochemistry

Tissue microarrays (TMA) were constructed using four 1.0 mm cores each. These were immunostained for neuroendocrine markers, cell-cycle regulators as well as targeted therapy-related markers. The antibodies used in this study and corresponding clones, vendors and titres used have been listed in Supplementary Table 1, http://links.lww.com/PAT/A35. Immunostained TMAs were subjected to validation, to determine whether multiple cores accurately represented the underlying tumour. A total of 119 (of 122) UC and 76 (of 79) HGNEC for which formalin, fixed paraffin embedded tissue blocks were available were validated and immunostaining results are reported only for these cases. Positive staining was further graded as 1+ (weak staining), 2+ (moderate staining) and 3+ (intense staining).

Morphological analysis

Morphometric analysis to compute the mean cell and nuclear size (area) was performed using the software cellSens Dimension (Olympus Soft Imaging Solutions, Germany). For each specimen, a representative image acquired at 400× magnification was analysed. Subsequently, a total of 10 representative cells were quantified for each image to yield a mean value. Total number of specimens analysed were 122 (UC) and 79 (HGNEC). Nuclear size for lymphocytes (Ly; $n = 35$) served as an internal control. For this analysis, HGNEC were further subclassified as small cell ($n = 68$), mixed-NEC ($n = 6$) and LCNEC ($n = 5$). Furthermore, this analysis was repeated after dividing the HGNEC group into those that were initially misdiagnosed as UC (M-HGNEC; $n = 37$) and those that were correctly diagnosed as HGNEC (C-HGNEC; $n = 42$). Similarly, proliferative index was calculated by acquiring a representative image of Ki-67 labelled cells at 400× magnification for each case, followed by a manual quantification of Ki-67 positive cells using ImageJ, version 1.47 (National Institutes of Health, USA).

Statistical analysis

Clinicopathological comparisons of HGNEC and UC were performed using the Wilcoxon test for continuous variables and Chi-square or Fisher's exact test for

Table 1 Clinicopathological features of patients with HGNEC and matched patients with UC

Patient characteristics	UC ($n = 122$)	HGNEC ($n = 79$)	p value (UC vs HGNEC)	Small cell ($n = 68$)	Mixed-NEC ($n = 6$)	LCNEC ($n = 5$)
Age	($n = 122$)	($n = 79$)	0.04	($n = 68$)	($n = 6$)	($n = 5$)
Mean (SD)	67.8 (9.9)	70.4 (9.4)		70.5 (9.7)	67.7 (7.7)	71.8 (9.3)
Range	(44.0-90.0)	(39.0-87.0)				
Gender	($n = 122$)	($n = 79$)	0.29	($n = 68$)	($n = 6$)	($n = 5$)
Female	24 (19.7%)	11 (13.9%)		10 (14.7%)	0 (0.0%)	1 (20.0%)
Male	98 (80.3%)	68 (86.1%)		58 (85.3%)	6 (100.0%)	4 (80.0%)
BMI	($n = 122$)	($n = 78$)	0.98	($n = 67$)	($n = 6$)	($n = 5$)
Mean (SD)	28.0 (5.5)	27.9 (4.6)		27.6 (4.5)	31.7 (5.9)	27.6 (3.3)
ECOG status	($n = 122$)	($n = 78$)	0.61	($n = 67$)	($n = 6$)	($n = 5$)
0	92 (75.4%)	64 (82.1%)		55 (82.1%)	5 (83.3%)	4 (80.0%)
1–3	30 (24.6%)	14 (17.9%)		12 (17.9%)	1 (16.7%)	1 (20%)
Pathological stage	($n = 122$)	($n = 79$)	0.99	($n = 68$)	($n = 6$)	($n = 5$)
T1	6 (4.9%)	4 (5.1%)		4 (5.9%)	0 (0.0%)	0 (0.0%)
T2a	5 (4.1%)	3 (3.8%)		3 (4.4%)	0 (0.0%)	0 (0.0%)
T2b	12 (9.8%)	6 (7.6%)		3 (4.4%)	3 (50.0%)	0 (0.0%)
T3a	34 (27.9%)	23 (29.1%)		19 (27.9%)	1 (16.7%)	3 (60.0%)
T3b	44 (36.1%)	28 (35.4%)		27 (39.7%)	1 (16.7%)	0 (0.0%)
T4a	16 (13.1%)	13 (16.5%)		10 (14.7%)	1 (16.7%)	2 (40.0%)
T4b	5 (4.1%)	2 (2.5%)		2 (2.9%)	0 (0.0%)	0 (0.0%)
N	($n = 122$)	($n = 78$)	0.70	($n = 67$)	($n = 6$)	($n = 5$)
NX	8 (6.6%)	4 (5.1%)		4 (6.0%)	0 (0.0%)	0 (0.0%)
N0	74 (60.7%)	50 (64.1%)		41 (61.2%)	4 (66.7%)	5 (100.0%)
N1–3	40 (32.7%)	24 (30.8%)		22 (32.8%)	2 (33.3%)	0 (0.0%)
Disease recurrence	($n = 122$)	($n = 79$)	0.21	($n = 68$)	($n = 6$)	($n = 5$)
Absent	62 (50.8%)	33 (41.8%)		28 (41.2%)	3 (50.0%)	2 (40.0%)
Present	60 (49.2%)	46 (58.2%)		40 (58.8%)	3 (50.0%)	3 (60.0%)
Bladder cancer specific death	($n = 122$)	($n = 79$)	0.47	($n = 68$)	($n = 6$)	($n = 5$)
Absent	51 (41.8%)	29 (36.7%)		24 (35.3%)	3 (50.0%)	2 (40.0%)
Present	71 (58.2%)	50 (63.3%)		44 (64.7%)	3 (50.0%)	3 (60.0%)
Time to last FU (amongst alive)	($n = 18$)	($n = 9$)	0.15	($n = 8$)	($n = 1$)	($n = 0$)
Mean (SD)	12.7 (5.8)	9.8 (5.3)		9.1 (5.3)	14.8	–

BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; FU, follow up; HGNEC, high grade neuroendocrine carcinoma; LCNEC, large cell neuroendocrine carcinoma, mixed-NEC, mixed neuroendocrine carcinoma; UC, urothelial carcinoma.