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ORIGINAL ARTICLE

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Small-cell carcinoma of the urinary bladder. A clinico-pathological study of ten cases

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Abstract Small-cell carcinoma (SCC) of the urinary bladder is an infrequent neoplasia accounting for 0.5% of all tumors located at this level. There is a predilection for males over females with a 4:1 proportion and a median age of 66 years. In most cases, the initial diagnosis is made at the metastatic or progressive stage of the disease. For this study, we collected ten cases of SCC of the urinary bladder, diagnosed over a period of 16 years, to describe the morphological and immunocytochemical characteristics of these infrequent neoplasia. In all cases, clinical data such as age at presentation, personal background, clinical symptoms, stage, treatment, clinical outcome and present status were available. Primary antibodies included chromogranin, neuron-specific enolase, synaptophysin, PGP 9.5, HNK-1, cytokeratin 34βE12, cytokeratin 20, thyroid transcription factor-1 (TTF-1), c-erbB2 (CB-11), p53 (DO7), and Ki67 (MIB-1). In addition to the expression of neural/neuroendocrine markers, immunostaining for p53 and c-erbB2 was found in 80% and 50% of cases, respectively. In this paper, we confirm the aggressive course of the neoplastic disease. The expression of c-erbB2 in 50% of cases opens up hypothetical new possibilities for the use of immunotherapy in such cases.

Keywords Small-cell carcinoma · Urinary bladder · Immunohistochemical · Oat-cell carcinoma · Survival · c-erbB2

Introduction

Small-cell carcinoma (SCC) of the urinary bladder is an infrequent neoplasia accounting for 0.5% of all tumors located at this level. A predilection for males over females with a 4:1 proportion and a median age of 66 years [4, 22] are characteristics of this neoplasia. The clinical presentation includes hematuria and is less often found with pain or obstructive symptoms. In most cases, the initial diagnosis is made at the metastatic or progressive stage of the disease. Exceptionally, paraneoplastic symptoms derived from adrenocorticotrophin secretion [22, 33], hypercalcemia [8, 12, 22, 34, 35] or hypophosphatemia are observed [12]. The main diagnosis is based upon the histopathological features, consisting of a diffuse growth pattern of small cells with a highly condensed chromatin, the immunodetection of neuroendocrine markers and the presence at the ultrastructural level of dense core neurosecretory granules [13]. In addition, several reports indicate the possibility of diagnosing this SCC in urinary cytology specimens [1, 3, 9, 25, 26].

From the molecular genetic point of view, several chromosomal abnormalities have been described. Terracciano et al. [41] reported deletions in 10(q), 4q, 5q and 13 q, as well as gains in 8q, 5p, 6p and 20q, and genetic amplification in 1p22–32, 3q26.3, 8q24 y 12q14–21 in ten tumors analyzed using comparative genomic hybridization. Leonard et al. [24] described a monosomy 9, homozygous deletion of P16 and trysomy 7 using conventional karyotyping and mutations of p53 as being event frequent.

The clinical behavior is very aggressive, with early lymph-node metastasis and liver, bone or peritoneal cavity deposits [8]. The prognosis is based upon the clinical staging and the therapeutic strategy, but a high percentage of deaths (68.7%) are seen within 2 years of follow-up [42]. The purpose of this paper is to communicate a number of new cases of this entity, supported with immunohistochemistry and focusing on possible therapeutic targets.

Materials and methods

For this study, we collected ten cases of SCC of the urinary bladder, diagnosed over a period of 16 years and retrieved from the files of the Department of Pathology of the University Clinic Hospital of

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Table 1 Clinical data, outcome and present status for all cases. *SURG* radical cystectomy, *TUR* transurethral resection, *CT* chemotherapy, *RT* radiotherapy, *DOD* died of disease, *A* alive, *Survival* minimal survival in the last control

Mean age/sex	Onset	Stage initial	Metastases	Treatment	Survival
70 Years/male	Hematuria	-	-	TUR	-/DOD
47 Years/male	Hematuria Polaquiuria Tenesm	PT3bN2M0	Bone	SURG+CT+RT	12 Months/DOD
72 Years/male	Hematuria	-	Liver Bone	SURG+CT+RT	16 Months/DOD
52 Years/male	Hematuria	pT3N3M0	Lymph nodes	SURG+CT	+16 Months/A
82 Years/male	Hematuria Dysuria Polaquiuria Incontinence	pTxN2M0	Lymph nodes	TUR	+2 Months/-
57 Years/male	Hematuria	pT4aN2M0	Lymph nodes	SURG+CT	+3 Months/-
82 Years/male	Urinary discomfort	-	-	TUR	3 Months/DOD
66 Years/male	Hematuria	-	Lung Liver Bone	SURG+CT+RT	15 Months/DOD
74 Years/female	Hematuria Hipogastric Slowness	-	Lymph nodes Liver Mediastinum Soft tissue	TUR+RT	6 Months/DOD
52 Years/male	Hematuria	pT3N0M0	-	SURG+CT	+16 Months/A

Valencia. The material was formalin fixed and paraffin embedded, and the sections were immunostained using the avidin-biotin peroxidase method. Antigen retrieval was obtained using heating in autoclave (1.5 ATM, 3 min.) and citrate buffer pH 6.0.

Primary antibodies included chromogranin A (Biomeda 1/50), neuron-specific enolase (Biogenex 1/50), synaptophysin (Boehringer Mannhein 1/20), PGP 9.5 (Biomeda 1/50), HNK-1 (ATCC 1/10), cytokeratin 34βE12 (Dako 1/50), cytokeratin 20 (Biogenex 1/50), thyroid transcription factor-1 (TTF-1) (Dako 1/50), c-erbB2 (CB-11, Novocastra 1/50), p53 (DO7, Dako 1/50) and Ki67 (MIB-1, Dako 1/50). In all cases, clinical data such as age at presentation, personal background, clinical symptoms, stage, treatment, clinical outcome and present status were available (Table 1).

Results

Clinical aspects

The mean age of the patients was 65 years (47–82 years). A clear predominance of males in a proportion 10:1 was observed. The existence of several types of antecedents, such as smoking (5/10), hypercholesterolemia (4/10), benign neoplasms (Warthin tumor, 1/10) and larynx epidermoid carcinoma (1/10) were identified. All but two patients presented metastatic disease on initial diagnosis at the following locations: lymph nodes (4/10), liver (3/10), bone (2/10) and soft tissue (1/10).

Pathological findings

Macroscopically, these tumors ranged in size from 2-cm polipoid lesions to 10-cm solid neoplasms (Fig. 1). The overlying mucosa was commonly ulcerated. Microscopically, the tumors displayed a uniform diffuse or organoid growth pattern and were constituted of solid nests of intermediate or small cells, showing scarce cytoplasm, round nuclei with granular dense chromatin and no evident nucleoli (Fig. 2). Less numerous intermediate cells displayed large visible cytoplasm and a voluminous nuclei with granulous dense core chromatin. Other common features found were extensive necrosis and numerous mitosis (more than 28 per 10 high-power field). Azzopardi artifact was also evident.

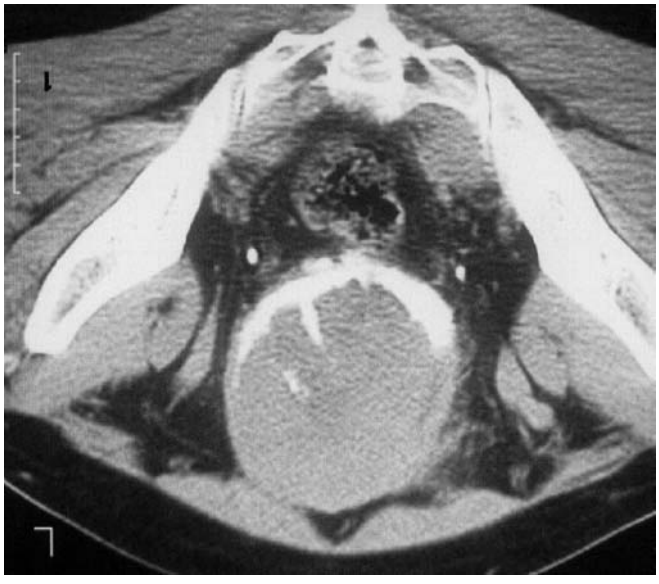

Fig. 1 Computed tomography scan showing a solid neoplasm invading the bladder cavity

The immunohistochemical analysis revealed the presence of two or more neuroendocrine markers in each case. Chromogranin was positive in 50% of cases (5/10) (Fig. 3), NSE in 100% (10/10), synaptophysin in 40% (4/

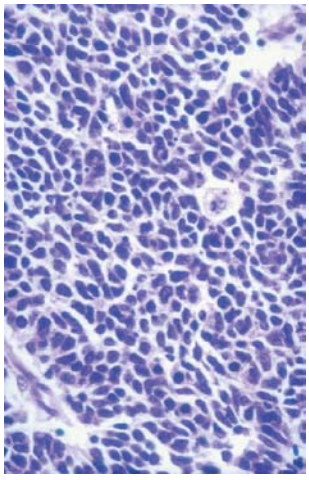

Fig. 2 Solid nest of small cells showing scarce cytoplasm, round nuclei with granular dense chromatin (hematoxylin and eosin ×40)

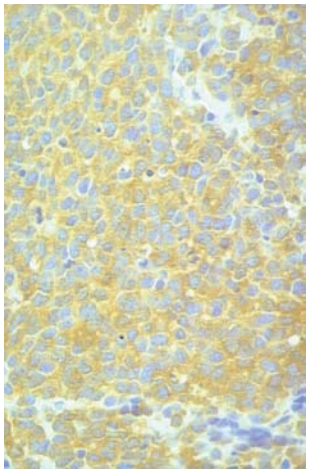

Fig. 3 Intense cytoplasmic expression of chromogranin (×40)

10), PGP 9.5 in 40% (4/10) and HNK in 50% (5/10). In addition, 34βE12 cytokeratin expression was observed in 40% of cases (4/10), whereas CK 20 was negative in all cases. Finally, 20% of cases (2/10) showed expression of TTF-1. The immunohistochemical results are summarized in Table 2.

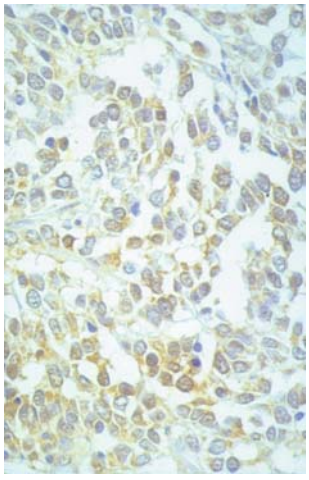

Fig. 4 A typical membranous c-erbB2 immunostaining pattern (×40)

Interestingly, c-erbB-2 immunostaining was observed in 50% of cases with a typical membranous pattern (Fig. 4). Moreover, p53 nuclear expression was found in 80% of cases (8/10). The proliferative index measured with Ki 67 (MIB 1) expression varied from 15% to 70%, with a mean of 33%.

Discussion

It is important that small-cell neuroendocrine carcinomas of urinary bladder be clearly distinguished from their non-neuroendocrine counterparts because of differences in treatment and prognosis [8]. The clinical outcome of this type of carcinoma is very poor, although a relatively better survival of patients has been reached in some series [8].

The present clinical records of our cases are similar with respect to age (65 years), and initial symptoms [17, 27] to those indicated in the literature [22, 42]. Metastatic spread to the lymph nodes, liver, bone and soft tissue as

Table 2 Immunohistochemical study. *CHROM* chromogranin, *NSE* neuron-specific enolase, *SYN* synaptophysin, *CK P* cytokeratin 34βE12, *CK 20* cytokeratin 20, *TTF-1* thyroid transcription factor-1, + <25% positive cells, ++ 25–75% positive cells, +++ >75% positive cells, *C-erbB-2 0/+* cases negatives, *++/+++* cases positives (Dako system)

Case	CHROM	NSE	SYN	PGP	HNK1	CK P	CK 20	TTF-1	C-erb	P53	Ki67
1	0	++	+	++	+++	0	0	0	+	90%	45%
2	++	+++	0	0	0	+++	0	0	+	0	15%
3	+++	+++	0	+++	+	0	0	0	0	50%	50%
4	0	++	+	0	++	0	0	0	0	60%	40%
5	0	+++	0	++	0	+	0	0	0	30%	30%
6	+++	+++	+	0	+	0	0	+++	0	70%	70%
7	0	+++	0	0	0	0	0	0	++	90%	20%
8	++	+++	+	+++	0	+++	0	0	++	90%	20%
9	+++	+++	+++	0	0	0	0	+	0	25%	30%
10	0	++	0	0	+	++	0	0	++	0	15%

the locations preferentially involved is also in agreement with previous reports [33].

Regarding treatment, 30% of patients (3/10) were treated exclusively using TUR (transurethral resection) , with a survival of 2–5 months (death due to obstructive uropathy). The survival improved up to 14 months in those cases (3/10) treated using cystectomy, CT (chemotherapy) and RT (radiotherapy) in combination. The mean survival of our patients was 8.77 months, lower than that reported in some series [7, 8, 18, 25] but consistent with other published survivals of 6 months [23, 30], 6.9 months [20] and 8 months, respectively [9]. In addition to the clinical aggressiveness, the major diagnosis came from histology and the immunohistochemical detection of neuroendocrine markers.

All cases except one presented at least two neuroendocrine markers confirming previous reports. Iczkowski et al. [43] demonstrated a high expression of chromogranin (65%), synaptophisin (76%) and NSE (93%). Additionally, Van Hoeven et al. [26] showed expression of NSE in 11 of 11 cases and synaptophisin in 2 of 11 cases, whereas chromogranin was detected in only 2 of 13 cases and Leu 7 in 2 of 7 cases, respectively. Grignon et al. [17] detected the following immunoprofile: NSE in 10 of 10, chromogranin in 8 of 9, serotonin in 7of 9 and s-100 in 4of 10 cases. Helpap and Kollermann [21] showed that the expression of chromogranin was highest in low-grade neuroendocrine tumors and lowest in high-grade small-cell neuroendocrine carcinomas. Cytokeratin immunostaining is a common feature in SCC [17, 43]. Moreover, 34βE12 cytokeratin expression has also been reported in these tumors [22, 32, 44]. In this study, we detected such expression in 40% of our cases. Controversial results regarding CK 20 expression in SCCs have been published. In our study, all the cases proved negative for CK 20 in agreement with the results published by Ordoñez et al. [11] and Helpap [20]. In contrast, Cheuk et al. [2] reported expression of CK 20 in 4% of extrapulmonary SCCs.

TTF-1 is a marker that has been described in extrapulmonary SCC [2, 11, 37]. In this study, we saw expression in two cases. This data limits the value of this marker for establishing the differential diagnosis between primary SCC of the bladder and possible bladder metastasis from a pulmonary SCC. The human epidermal-growth-factor receptor-2 (c-erbB-2) is overexpressed and amplified throughout a broad spectrum of tumor types in breast, bladder, ovarian, salivary gland, endometrium, pancreas and lung [37]. C-erbB-2 is implicated in disease initiation and progression, is associated with poor prognosis and may also predict the response to chemotherapy and hormonal therapy. Above all, its expression is considered to be a negative prognostic indicator in metastatic breast carcinoma. A relationship between high expression of c-erbB-2 and poor survival has been reported in breast cancer. In these cases, clinical phase-II and -III trials have demonstrated the efficacy of the humanized anti-c-erbB-2 Mab, Trastuzumab (Herceptin), both as a single agent and in combination with chemotherapy, in c-erbB-2-positive metastatic breast cancer patients [10, 29, 38]. The expression of c-erbB-2 in SCC of the bladder has not previously been reported. In our analysis, we demonstrated high expression of c-erbB-2 in 50% of the cases. This expression should be used as a prognostic indicator in bladder SCC (as already confirmed in transitional tumors) [5] and, moreover, opens up a new method of planning clinical trials and assessing the therapeutic role of Trastuzumab in SCC of the bladder. A recent study found an inverse relationship between c-erbB2 expression and survival in transitional-cell carcinoma of the urinary bladder [28]. The mean survival of our patients was 4 months in positive c-erbB-2 cases and 12.83 months in negative c-erbB-2 cases. Accordingly, we confirm an inverse relationship between c-erbB-2 expression and survival in SCC of the urinary bladder. Currently, fluorescence in situ hybridization analysis of c-erbB-2 gene amplification is in progress, and, although the results are not yet available, they will serve for a future publication.

Mutations of the tumor suppressor gene p53 of chromosome 17 have already been reported in bladder SCC [24, 28]. Among our cases, we described a high expression of p53 (63%), possibly representing a poor prognostic factor as well as an indicator of neoplastic progression. Mutations of the p53 suppressor gene have been found in urothelial carcinoma [6, 14, 16, 19, 31, 36, 39, 40, 45]: several studies have demonstrated a positive correlation between p53 abnormalities and higher tumor stage [14, 16, 40, 45]. Sarkis et al. [36] reported a higher risk of progression in patients with pT1 tumors with abnormal p53 protein expression than in those lacking the expression. Esriget et al. [15] observed that p53 overexpression correlated significantly with recurrence and crude survival, and, in multivariate regression analysis, it was a factor independent of pathological stage and histological grade. For muscle-invasive tumors (pT2 y pT3a) the 5-year survivals were 79% and 64% for p53 negative cases and 30% and 22% for p53 positive cases, respectively.

In conclusion, we believe that it is very important in bladder cancer to identify the neuroendocrine differentiation as a prognostic indicator to choose the appropriate therapy. Thus, the prognosis could then be improved with multidisciplinary treatment [17, 22]. Moreover, the overexpression of c-erbB-2 in cases of SCC opens up new methods for the application of immunotherapy in these infrequent and aggressive neoplasms.

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