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ORIGINAL ARTICLE

Clinicopathologic and Immunohistochemical Study of Combined Small Cell Carcinoma and Urothelial Carcinoma Molecular Subtype

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Abstract

Muscle invasive bladder cancer, an aggressive disease with heterogeneous molecular profiles, has recently been subclassified into three major molecular subtypes -basal, luminal and "p53-like" urothelial carcinomas (UCas), which bear prognostic and therapeutic implication. Similar to breast cancer, basal and luminal subtype UCas are designated by basal (CK5/14) and luminal (CK20) markers. The "p53-like" subtype presents with wild-type p53 gene with upregulated p53 pathways and is implicated in chemoresistance. Urinary bladder is one of the most common primary sites of extrapulmonary small cell carcinoma (SmCC). Bladder SmCC frequently coexists with UCa; however, the relation of SmCC with specific UCa molecular subtypes has not been studied. The aim of this study is to investigate the clinicopathology and immunophenotypes of the combined SmCC and UCa molecular subtypes. A total of 22 combined SmCC and UCa cases were studied for the clinicopathology and immunohistochemical (IHC) profiles by luminal and basal cell markers as well as Her2/Neu and p53. Our results demonstrated that all the urinary bladder SmCCs were associated with high grade UCas. They were more commonly seen in older male patients with a smoking history and had a poor prognosis. Based on the reported molecular subtyping, the UCas could be immunohistochemically subclassified into luminal, basal, dual and null types, which showed different clinicopathologic and IHC features. Compared to non-SmCC associated UCa, the subtypes of UCa in the combined SmCCs and UCas were characterized by: 1) Although overall luminal type was still relatively more common in men, basal marker-expressing subtypes were significantly increased in incidence and were more common in women. 2) Her2/Neu overexpression was more commonly observed in luminal than basal cell marker-expressing UCas. 3) IHC overexpression of p53 was common in all the subtypes, with UCas and SmCCs sharing the same p53 expression pattern. Although limited by relatively a small number of cases, the results of this study will enhance our understanding of the combined SmCC and UCa entity and potentially lead to a future therapeutic management.

Keywords Urothelial carcinoma · Small cell carcinoma of urinary bladder · Molecular subtype · Immunohistochemistry

Introduction

Bladder cancer is the most common malignancy of the urinary tract. More than 90% of bladder cancers are urothelial carcinoma (UCa). Low grade UCa frequently recurs but infrequently progresses to invasion [1]. High grade UCas, particularly muscle invasive bladder cancers (MIBCs), have a much less favorable prognosis with a high frequency of progression to metastasis and a less than 50% five-year overall survival [2]. At present, the standard of care for patients with localized MIBC is radical cystectomy preceded by cisplatin-based neoadjuvant chemotherapy [3]. However, responses to chemotherapy are seen in only 40%–60% of cases and metastatic disease is frequently detected at the time of surgery [3]. The underlying mechanisms for such differential chemotherapeutic responses are yet unknown. Recent studies have shown that MIBC is an aggressive disease with heterogeneous molecular profiles [4]. Choi et al. (2014) used whole-genome gene expression profiling to identify three major molecular subtypes of MIBC, which they termed basal, luminal and

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"p53-like" UCas. Their study demonstrated that such subtyping bore prognostic and therapeutic implication. The basal and luminal subtypes are designated by the markers similarly used for basal and luminal-type breast cancers [5]. Basal MIBCs characteristically express CD44, cytokeratins 5, 6, and 14 and lack cytokeratin 20 expression, while the luminal MIBCs have the converse expression of these markers [4]. Such differential expression of cytokeratins 5/14 and 20 reflects the hierarchy of urothelial cell differentiation with the least differentiated cells located in the basal layer [6]. In addition, they also depicted a "p53-like" subtype UCa, which expresses luminal biomarkers. This type of UCa is distinguished by an activated wild-type p53 gene signature with upregulation of the p53 pathway genes and has been implicated in chemoresistance in MIBC [4].

Small cell carcinoma (SmCC) is an aggressive tumor that is most commonly reported in the lung, where it accounts for 20% of cancers [7]. Extrapulmonary SmCC is not uncommon and can arise anywhere in the body except the central nervous system. Although the urinary bladder is one of the most common primary sites, SmCC accounts for less than 1% of all the primary bladder cancers [8]. Similar to those in the lung, more than 60% of SmCCs of the urinary bladder will present with metastasis at the time of diagnosis and the prognosis is dismally poor [8–10]. SmCC of urinary bladder frequently coexists with UCa [8, 10]; however, the relation of SmCC with specific UCa molecular subtypes has not been studied. The aim of this study is to investigate the clinicopathology and immunophenotypes of the combined SmCC and molecular subtypes of UCa.

Methods and Materials

Tissue Samples

Histologic criteria for diagnosis of urinary bladder SmCC were the same as those used for pulmonary SmCC described by the WHO classification system [11]. Briefly, diagnosis of bladder SmCC was rendered solely on morphologic grounds with immunohistochemistry documenting neuroendocrine differentiation. Diagnosis and grading of urothelial carcinoma was made according to 2016 WHO classification criteria [12]. Tumors were staged based on American Joint Committee on Cancer staging system for urinary bladder tumor [13].

A total of 22 combined SmCCs and UCas were identified from the files accessioned between 2007 and 2016 in the Departments of Pathology, University of Rochester and Lenox Hill Hospital, New York. The UCa was present as either high grade papillary UCa/carcinoma in situ (CIS) or invasive carcinoma, or both. Pathologic parameters, patient history and demographics as well as treatment and survival data were gathered and recorded for clinical-pathologic correlation.

Immunohistochemical Analysis and Classification of UCa

Immunohistochemistry was performed using Dako automated system with the same standard protocol as used for clinical samples along with appropriate positive and negative controls. Immunohistochemical markers included CK5, CK14, CK20, HER2, p53 and / or neuroendocrine markers (Chromogranin, Synaptophysin and CD56). UCa components were subclassified into luminal and basal types based on their phenotypes in a molecular study [4]. Briefly, UCa reactive with CK5 and /or CK14 but non-reactive with CK20 was classified as basal cell subtype and those reactive with CK20 but non-reactive with CK5 or CK14 was classified as luminal cell subtype. The UCa reactive with both CK5/14 and Ck20 or neither CK5/14 or CK20 was classified as unclassified type (dual type and null type, respectively). Her-2/neu was scored according to the standard criteria used for breast cancer [14]. Briefly, 0 (negative) was defined as incomplete and / or faint/barely perceptible membrane staining and within ≤10% of the tumor cells. 1+ (negative) was defined as incomplete and/or faint/barely perceptible membrane staining and within >10% of the tumor cells. 2+ (weakly positive/equivocal) was defined as weak/moderate membrane staining and within >10% of the tumor cells or complete and circumferential membrane staining that is intense and within ≤10% of the tumor cells. 3+ (strongly positive) was defined as circumferential membrane staining that is complete, intense in more than 10% of the tumor cells. The staining of all the other immunohistochemical markers were recorded as positive (+) if there were more than 10% of staining tumor cells or negative (−) if there were less than 10% of staining tumor cells. The differences in the expression and other parameters between the molecular subtype groups were analyzed by two-tailed t-test. Significant difference was defined by $p < 0.05$.

Results

Clinicopathology of the Combined SmCCs and UCas

All the urinary bladder SmCC cases identified during the aforementioned 10-year-period were associated with high grade UCa. There were neither pure SmCC cases nor SmCC cases in association with low grade UCa. Patients' ages ranged from 59 to 96 (mean 74.4, median 73). Males were more commonly affected than females (male to female

ratio: 8:3). Smoking history was noted in the majority of patients (21/22, 96%). 21/22 (96%) patients presented with muscle invasive disease (≥pT2). Excluding the two patients who were lost to follow up and three patients who were still alive, the years of survival ranged from less than 1 year to 6 years (mean: 2.3 years, median 1 year). 10/17 (56%) patients died within 1 year. 3/17 (18%) patients lived beyond 5 years. 11/22 (50%) patients had Cisplatin-based chemotherapy and 9/22 (41%) patients had no chemotherapy. Treatment history was unknown in 2/22 (9%) patients. Based on the limited number of cases and follow up data, no significant difference in clinical response was noted among the following subclassified groups. The amount of SmCC component in the tumor ranged from 5 to 95%. 95% (20/22) of the cases were associated with conventional UCa, only 9% (2/22) UCas presented with foci of other morphologic variant (1 micropapillary and 1 sarcomatous). With available follow-up data, 11/15 (73%) patients had metastasis and 14/17 (82%) of the deaths were cancer-related. The clinicopathologic results are summarized in Table 1.

Immunohistochemical Profiles of the Combined SmCCs and Molecular Subtype UCas

Among the UCas in the 22 cases of combined SmCCs and UCas, 7 were of basal cell type, 9 were of luminal type. There were 6 unclassified cases, of which 2 expressed both basal cell and luminal cell markers (dual type UCa) and 4 expressed neither basal cell nor luminal cell markers (null type UCa). Basal cell type UCas accounted for 32% (7/22), luminal type 41% (9/22) and unclassified type 27% (6/22) (null type 18% (4/22) and dual UCa 9% (2/22)). The mean age was 72.4 for basal type, 75 for luminal type, 74 for dual type, and 76.3 for null type. There was no significant age difference between these groups. The male to female ratio was 8:1 in luminal type, 4:0 in null type, 3:4 in basal cell type, and 1:1 in dual type UCas. The cytokeratins (CK20, CK5 and CK14) were mostly lost in the associated SmCC.

All luminal UCas showed overexpression of Her2/Neu (9/9 (100%) with score ≥ 2+), as did the dual UCas (2/2 (100%) with score 3+). The basal cell type and null type UCa showed

Table 1 Clinicopathology and immunohistochemical profiles of the combined small cell carcinomas and urothelial carcinomas

Case	CK20 UC	CK20 SmCC	CK5 UC	CK5 SmCC	CK14 UC	CK14 SmCC	Her2 UC	Her2 SmCC	P53 UC	P53 SmCC	% SmCC	Chemo-therapy	Survival (years)	Age/sex	Procedure and pTN	Metastasis and site
1	+	+f	–	–	–	–	3+	–	–	–	50	No	<1	95 M	TURB,T2	Liver
2	+	–	–	–	–	–	2+	–	+	+	5	No	<1	96 M	TURB,T2	NA
3	+	–	–	–	–	–	3+	1+	+	+	90	Yes	6	59 M	T1 N0	NA
4	+	–	–	–	–	–	3+	–	–	–	80	Yes	5	62 M	T2 N0	NA
5	+	–	–	–	–	–	2+	–	+	+	50	No	2	74F	T3aN0	Abdominal soft tissue
6	+	–	–	–	–	–	3+	–	+	+	50	No	<1	66 M	TURBT, T2	NA
7	+	–	–	–	–	–	3+	–	+	+	60	unk	unk	73 M	TURBT, T2	No metastasis
8	+	–	–	–	–	–	3+	–	+	+	90	Yes	<1	81 M	TURBT, T2	Retroperitoneal LN
9	+	–	–	–	–	–	2+	–	+	+	70	Yes	alive	69 M	TURBT,T2	No metastasis
10	–	–	+	–	+	–	–	–	–	–	5	Yes	4	67F	TURBT, T2	Small bowel
11	–	–	+	–	+	–	–	–	+	+	90	Yes	5	77F	T2 N0	No metastasis
12§	–	–	+	–	–	–	3+	–	+	+	90	No	3	81F	T2 N1	Kidney, bone
13	–	–	+	–	+	–	–	–	+	+	20	Yes	<1	63 M	T2 N0	LN
14	–	–	+	–	–	–	–	–	+	–	<5*	unk	<1	71 M	T3bN0	NA
15	–	–	+	–	+	–	3+	–	+	+	90**	No	2	77F	T3bN0	Liver, small bowel, iliac LN
16	–	–	+	–	–	–	3+	1+	+	+	70	Yes	3	72 M	TURB, T2	LN
17	+	+f	+	+f	–	–	3+	–	+	+	15	No	<1	67 M	TURB, T2	NA
18	+	–	+	–	+	–	3+	–	+	+	90	No	alive	81F	TURBT, T2	No metastasis
19	–	–	–	–	–	–	1+	–	+	+	10	No	unk	80 M	T2 N0	NA
20	–	–	–	–	–	–	–	–	–	–	70	Yes	<1	62 M	TUTBT, T2	LN, liver
21	–	–	–	–	–	–	2+	–	+	+	95	Yes	<1	88 M	TURBT, T2	Adrenal, retroperitoneal LN
22	–	–	–	–	NA	NA	NA	NA	+	+	10	Yes	alive	75 M	TURBT, T2	Lung

* The UCa is associated with focal sarcomatous features; ** The UCa is associated with focal micropapillary features. § Nonsmoker

Unk, Unknown; UC, Urothelial carcinoma; SmCC, Small cell carcinoma; LN, Lymph node; NA, Not done / no data; +f, Focal positivity; M, Male; F, Female

significantly fewer number of cases with Her2/Neu overexpression; for the basal cell type, 3/7 (43%) had Her2/Neu overexpression (with score 3+) and 4/7 (57%) lacked Her2/Neu expression (score 0); for the null type UCa, only 1/4 (25%) had Her-2/neu overexpression (with score 2+). Except 2 cases (1 luminal type, and 1 basal cell type, each with negative Her2/neu expression (score 1+)), all the associated SmCC showed absence of Her-2/neu expression. p53 positivity was seen in the majority of the cases (18/22, 82%). The p53 positive rates for basal, luminal, null and dual types were 6/7 (86%), 7/9 (78%), 3/4 (75%) and 2/2 (100%), respectively. There seemed no significant difference of p53 positivity between these 4 groups. The UCa and SmCC showed exactly the same p53 immunohistochemical profile. A representative case of combined SmCC and luminal type UCa is illustrated in Fig. 1 and a representative case of combined SmCC and basal type UCa is illustrated in Fig. 2.

The immunohistochemical profiles of the 22 cases are shown in Table 1. The immunohistochemical characteristics and the distribution of patient sex and age among the UCa molecular subgroups subclassified by phenotypes are summarized in Table 2.

Discussion

The urinary bladder is the most common site for extrapulmonary SmCC. Bladder SmCC is mostly reported in older patients with a male predominance [8–10]. A smoking history is present in 50% to 70% of these patients [8–10]. It usually presents at an advanced stage at diagnosis with muscle invasion and frequent systemic metastasis [8–10]. In this series of combined SmCC and UCa, the mean age was 74.4 with a male to female ratio of 8:3. A smoking history was present in almost all (96%) of the patients. 95% (21/22) of our cases presented with muscle invasive disease. With available follow-up data, 73% (11/15) of the patients had metastasis and most of the patients (56%) died within the first year. These results largely support the existing clinicopathological literature of urinary bladder SmCC [8–10, 15].

Compared to the 70–80% of pure SmCC in its pulmonary counterpart, bladder SmCC is reported with another histologic subtype in approximately 40% to 50% of the cases [8–10, 15]. The mixed epithelial component is most commonly conventional UCa, followed by squamous cell carcinoma and adenocarcinoma. In our series, none of the bladder SmCC cases was

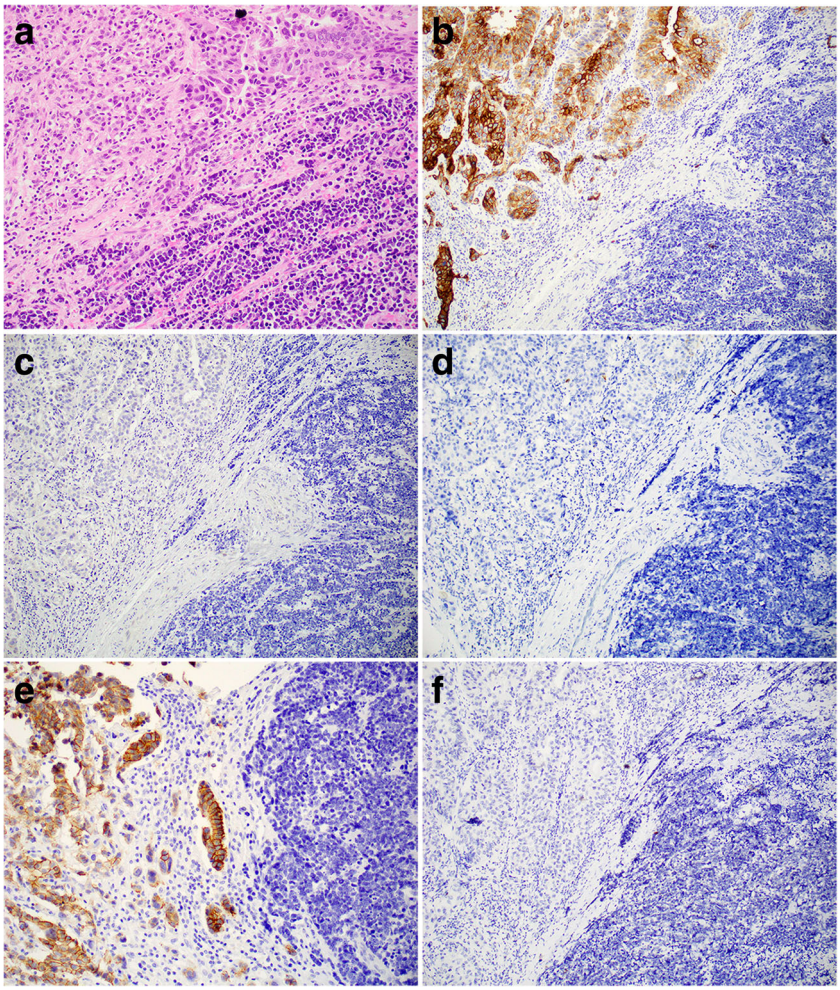

Fig. 1 Combined SmCC and luminal type UCa: **a** Hematoxylin-Eosin section- luminal type UCa (upper left) and SmCC (lower right). **b** CK20 inmunoreacting with UCa and non-reacting with SmCC. C and D. CK5 (**c**) and CK14 (**d**) non-reacting with UCa or SmCC. **e** Her2/Neu overexpression (score 3+) in UCa and no expression (score 0) in SmCC. **f** No p53 expression in UCa or SmCC

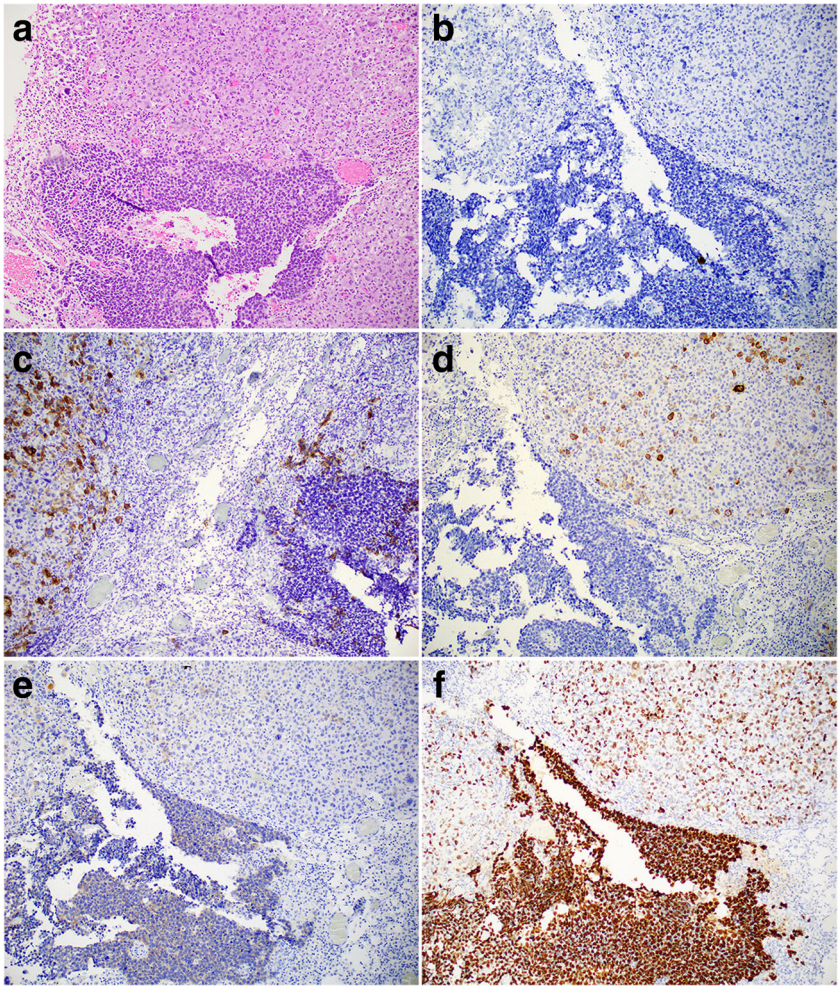

Fig. 2 Combined SmCC and basal cell type UCa: **a**. Hematoxylin-Eosin section- basal cell type UCa (upper) and SmCC (lower). **b** CK20 non-reacting with UCa or SmCC. **c** CK5 immunoreacting with UCa and sparsely with SmCC. **d** CK14 immunoreacting with UCa and non-reacting with SmCC. **e** No Her2/Neu overexpression (score 0) in UCa or SmCC. **f** p53 over-expression in both UCa and SmCC

pure and all the SmCCs were associated with high grade UCa, suggesting that SmCC in the bladder develops from the same molecular pathway as high grade UCa. 95% (20/22) of our cases were associated with pure conventional UCa, only 9% (2/22) presented with focal area of a morphologic variant.

Similar to breast carcinoma, based on the molecular features, bladder UCa has recently been subclassified into luminal and basal cell subtypes with each type expressing its respective cell markers. One recent study reported that, in non-SmCC associated UCas, luminal type UCa is 4 times more common than basal cell type UCa and basal type UCas are more commonly muscle invasive than luminal subtype UCas [16]. In this study of combined SmCC and UCa cases, we identified 4 groups of UCa. The luminal phenotype of UCa

Table 2 The immunohistochemical characteristics and the distribution of patient sex and age among the urothelial carcinoma subgroups sub-classified by phenotypes

	Basal cell type UCa CK5+/CK14+ and CK20-	Luminal cell type UCa CK20+ and CK5−/CK14-	Unclassified type of UCa	
			CK20- and CK5−/CK14- (null type)	CK20+ and CK5+/CK14+ (dual type)
Number of cases	7	9	4	2
HER2	3(3+), 4(−)	6(3+), 3(2+)	1 (2+), 1(1+), 1 (−), 1(unk)	2(3+)
P53	6(+), 1(−)	7(+), 2(−)	3(+), 1(−)	2(+)
Mean age	72.4	75	76.3	74
Sex	3 M, 4F	8 M, 1F	4 M, 0F	1 M, 1F

Unk, Unknown

was noted in 41% and basal cell type UCa in 32% of the cases, which, in comparison to a dominant luminal type reported in non-SmCC-associated UCa [16], indicates that basal cell type UCa is more common in combined SmCC and UCa cases, consistent with the proximity of basal cells to stem cells which are considered the precursor of SmCC. Nine percent of UCas expressed overlapping basal and luminal markers (dual type) and 18% expressed neither basal nor luminal markers (null type). Whether these 2 types of unclassified UCas belonged to specific groups of UCa with unique features or merely a result of tumor heterogeneity is yet unknown. There was no difference of muscular invasion status among the 4 subgroups, probably reflecting the already aggressive nature of these combined SmCC and UCa cases. There was no age difference between the 4 groups of UCas in our study. Overall there was a male predominance, however, considering that SmCC-associated UCas are reported more common in male (5:1 of male to female ratio), in contrast to luminal type and null type (male to female ratio: 8:1 and 4:0, respectively), basal cell marker (CK5/6 and CK14)-expressing groups (basal cell type and dual type) appeared more common in females (male to female ration: 3:4 and 1:1, respectively). Due to the limited number of cases, however, a larger cohort study is needed to verify this finding.

SmCC tended to lose the coexisting associated UC markers except for p53. It is reported that less than 10% of lung SmCCs react with CK20 [17]. Similarly, in our series, 9% of the bladder SmCC expressed CK20. Her2 protein overexpression and gene amplification are observed in various malignancies, including breast, ovary, stomach, colon, small intestine, and lung cancers [18–22]. At present, targeted anti-Her2 therapies are established for Her2 overexpressing/amplified carcinomas of the breast, stomach and esophagus. Recent works have also shown overexpression (score 2+ or 3+) or gene amplification of Her2 in approximately 10–80% of the UCa [23–27], and some studies have demonstrated the Her2/neu is an independent predictor for disease-related survival in muscle-invasive UCas [25–27]. Patients with Her2-amplified UCa could therefore potentially benefit from these therapies. From our study, Her2/Neu overexpression (score 2+ or 3+) was seen in 73% (16/22) of the UCas and overexpression of Her2/Neu strongly correlated with luminal marker (CK20)-expressing UCas (luminal type (100% (9/9)) and dual type (100%)). Although 50% (5 of 10) of bladder SmCC cases were reported to overexpress Her2/neu in one study [28], no Her-2/Neu expression in SmCCs (2/22, 9% score 1+) was found in our series. The luminal type of bladder cancer is reported to have a better prognosis and a good response to cisplatin-based therapy [4]. In this study, although luminal type UCas had a high incidence of Her2/Neu overexpression, they were less commonly seen in combined SmCC and UCa cases when compared with non-SmCC-associated UCa, which may need to be considered in the management of this disease. Future randomized clinical trials are needed to determine the responses of these different subtypes of UCas to chemotherapy, including personalized subgroup-based therapeutic regimens; for instance, luminal and dual subtypes of UCas, both of which exhibited, in this study, high prevalence of Her2 overexpression, may benefit from treatment with Herceptin and Cisplatin-based combinatorial therapy.

p53 gene mutation represents the most common genetic alteration in human cancers. p53 mutation has been associated with high grade, high stage, and poor prognosis in a variety of malignancies, including those of the lung, breast, stomach, prostate, and urinary bladder. Multiple case series have documented p53 overexpression in bladder SmCC, ranging from 37.5% (3 of 8) to 80% (8 of 10) of the cases studied in small case series [10, 29]. In our series, the p53 overexpression in SmCC showed the same patterns as its associated UCa. p53 overexpression was observed in 77% (17/22) of the SmCC and its associated UCa. There appeared no correlation between p53 overexpression and poorer prognosis, again probably because of their aforementioned already poor prognosis. It should be noted that immunohistochemical overexpression of p53 can be caused by p53 mutation (false p53 overexpression resulting from prolonged p53 protein stability) or wild type p53 gene overexpression (the so-called "p53-like" Cisplatin-based chemotherapy-resistant UCa group (4)); therefore, further p53 gene expression study is warranted in differentiating these two groups for the purpose of selection of a personalized therapy.

It was previously speculated that both pulmonary and extrapulmonary SmCCs are derived from the cells of the amine precursor uptake and decarboxylation system in neural crest. However, the frequent association of SmCC with UCa in urinary bladder and the overall similar molecular profiles and genetic alterations between SmCC and the associated UCa suggest a multipotent common stem cell origin [7–9] rather than from a specific neuroendocrine precursor cell. The immunohistochemical results of our study were also in line with the multipotent stem cell theory.

Urinary bladder SmCC responds to the same chemotherapy regimens used in pulmonary SmCC; a definitive treatment, however, for the combined SmCC and UCa is not yet established. Combination of aggressive systemic chemotherapy followed by local therapy (cystectomy or radiation therapy) has been reported to be the most effective therapeutic approach for clinically localized bladder SmCC [9, 15, 30]. A better understanding of the features of SmCC and UCa molecular subtypes may facilitate future targeted treatment of this deadly disease.

In summary, combined SmCC and UCa is a highly aggressive bladder cancer with an overall male predominance, strong association with smoking and a dismal prognosis. Compared to non-SmCC associated UCa, the subtypes of the UCas in combined SmCCs and UCas were characterized by: 1)

Although overall luminal type was still more common in men, basal marker-expressing subtypes were significantly increased in incidence and were more common in women. 2). Her2/Neu overexpression was more commonly observed in luminal than basal cell marker-expressing UCas. 3). Immunohistochemical overexpression of p53 was common in all the subtypes of UCas, with UCas and SmCCs sharing the same p53 expression pattern. Although limited by a small number of cases, the results of such study will enhance our understanding of this lethal disease as well as potentially lead to a future therapeutic management.

Compliance with Ethical Standards

Conflict of Interest None.

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