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REVIEW ARTICLE

Small cell carcinoma of the bladder: the characteristics of molecular alterations, treatment, and follow-up

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Abstract

Small cell carcinoma of the bladder (SCCB) is a rare disease associated with high invasiveness and mortality. Histologically, SCCB is difficult to distinguish from small cell lung cancer (SCLC); however, it shares more similar molecular alterations with urothelial carcinoma (UC). As a result, now, the widely accepted theory about the cells of origin is that SCCB and UC probably have a common clone origin. Even the former probably comes from a preexisting UC. At present, given its rarity, early diagnoses, treatments, and follow-ups are not well established, which are vital to patients with SCCB. Inspirationally, in recent years, with the development of molecular diagnostic methods, molecular alterations of SCCB have been understood partially, which are propitious to excavate new potential therapeutic strategies and establish sound follow-ups. Therefore, the future will be light for patients with SCCB.

Keywords Small cell carcinoma of the bladder · Urothelial carcinoma · Small cell lung cancer · Molecular alterations · Treatment · Follow-up

Introduction

Bladder cancer is the 10th most common form of malignancy worldwide. In 2018, 549,000 new cases were diagnosed and 200,000 cases died of bladder cancer [1]. Neuroendocrine cancer of bladder (NECB) represents approximately less than 1% of all urinary bladder malignancies. SCCB is the major subtype of NECB and often mixes with urothelial components [2, 3]. Similar to UC, SCCB is more common in men (ratio of male to female = 3:1) and usually appears in the seventh to eighth decades of life. Painless gross hematuria and irritative voiding symptoms are most common initial signs [4, 5]. However, SCCB is usually advanced when it is diagnosed, with poorer prognosis compared to UC in the same stage [2].

Histologically, SCCB is similar to SCLC, but clinical manifestations are consistent with UC. Therefore, researchers devote to seeking for features of molecular alterations in SCCB.

Some studies found that SCCB had a high somatic mutational rate, which was mainly driven by APOBEC-mediated mutational process (accounted for 60 ± 23.7% of all somatic mutations) [6, 7]. The APOBEC-mediated mutational process was mainly C>G or C>T mutation. The similar mutational process was also observed in UC (accounted for 65.5% of all somatic mutations) [6–9]. SCLC harbored also a high mutational rate (7.4 protein-changing mutations per million base pairs) [10]. However, the mutational process was predominantly C: G>A: T transversions [11]. Hence, here, we summarize features of molecular alterations in SCCB by comparing with SCLC and UC, including cell cycle-related genes (TP53, RB1, CKDN2A, and MDM2), Telomerase

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reverse transcriptase (TERT) promoters, and chromosome modifiers. Meantime, we discuss potential clinical significance of these molecular alterations and current status of treatments and follow-ups in SCCB.

Molecular alterations in SCCB

The relevance of TP53, MDM2, RB1, CDKN2A

TP53 gene is located on chromosome 17p13 and codes the tumor suppressor protein, p53 protein. p53 protein participates in numerous cellular processes, containing cell cycle arrest, DNA repair, apoptosis, metabolism, autophagy, and so on [12, 13]. More than half of malignant tumors are associated with alterations of TP53 gene, and missense mutation of is mainly in the form of mutation [14–16]. TP53 gene mutations not only deprive p53 protein of its antitumor effect, namely loss-of-function, but may make some mutant p53 protein gain new ability to promote tumor formation, that is gain-of-function [17, 18].

Mutant p53 protein plays an important role in promoting tumorigenesis and development, but it needs the assistance of other molecules, such as MDM2, RB1, and CDKN2A.

It is recognized that MDM2 is a negative regulatory factor of p53 protein. The MDM2 gene is a pseudogene on chromosome 12, and encodes a MDM2 protein with distinctive E3 ubiquitin ligase effect, degrading p53 protein. In addition, MDM2 protein binds to the transactivated domain of p53 protein and inhibits its activity [19, 20]. Interestingly, p53 protein induces the transcription of MDM2 gene and prevents it from overexpressing in healthy conditions [21]. Therefore, the relationship of p53 protein and MDM2 protein is negative feedback. The balance is broken in tumors, leading to tumorigenesis [22].

Other than MDM2 gene, RB1 gene also implicates in tumor formation. RB1 gene is one of the most important tumor suppressor genes and encodes the pRb protein. pRb protein is also a negative regulator of cell cycle and limits the cell proliferation via inhibiting E2F transcription factors [23]. Meantime, pRb protein and p16 protein have negative feedback relationship. The p16 protein is encoded by tumor suppressor gene, CDKN2A gene. And it is a negative feedback role in cyclinD-CDK4/6-pRb-E2F cell cycle regulation pathway. The p16 protein prevents the phosphorylation of pRb by inhibiting CDK4/6, while hyperphosphorylated pRb can induce the expression of p16 protein and feedback inhibits the phosphorylation of pRb, thereby inhibiting the proliferation of the cells [24–26]. Mutations of CDKN2A gene, including homozygous deletion, loss of heterozygosity, and promoter abnormal methylation, can lead to cell proliferation out of control and promote tumor formation [27].

Molecular alterations and clinical significance of the TP53, MDM2, RB1, and CDKN2A genes in SCCB

Molecular alterations of the TP53, MDM2, RB1, and CDKN2A Genes in SCCB are also common. In a genomic analysis of 110 cases of SCLC, inactivating events of TP53 and RB1 were approximately 65% and 90%, respectively, and bi-allelic losses involving TP53 and RB1 were nearly 100% [28]. Consistent with SCLC, alterative events in TP53 and RB1 each affected up to 90% of patients and also had a high frequency of bi-allelic mutation of these two genes in SCCB [6, 29, 30]. UC also harbored the alteration of these two genes, however, the rate of which was lower than SCCB. Hence, mutations of TP53 and RB1 genes in small cell carcinoma were related to tissue [6].

However, in a review, researchers showed that the loss of TP53 and RB1 proteins may be the feature of invasive tumors rather than the basic characteristics of neuroendocrine tumors [31]. Similar to the above point of view, although dual loss of TP53 and RB1 was universal in SCCB, these genes were not sufficient for small cell phenotype [6, 8]. Therefore, other factors, other than the loss of TP53 and RB1, could contribute to the small cell phenotype. Furthermore, alterations of these genes allowed tumor cells getting lineage plasticity and made cells transit to alternative lineages, which building up a tolerance to drugs, escaping immune surveillance and so on [31].

Taken together, TP53 and RB1 are probably therapeutic targets for drug resistance. And further studies need to be conducted aiming to elucidate other driver gene mutations in SCCB.

In addition loss of TP53 and RB1, in SCCB, the inactivation of CDKN2A gene was mainly due to loss of heterozygous (frequency of LOH: 35–47%), as had been shown in UC (frequency of LOH: 30–47%) [32, 33]. By contrast, the deletion frequency of CDKN2A gene was 5% in SCLC [28]. CDKN2A gene deletion mutation was a predictor of increased aggressiveness and worse prognosis in UC [34, 35]. SCCB shares similar frequency of CDKN2A gene deletion mutation with UC, but it is undefined whether effects of CDKN2A gene deletion mutation are similar to that in UC. Recently, Chang et al. demonstrated that SCCB lacked CDKN2A gene deletion mutation ($P=0.02$) [6]. Thus, CDKN2A gene deletion mutation in SCCB is controversial. Further confirmation of the existence of CDKN2A gene deletion mutation and its role in SCCB studies will be useful in future.

Meantime, several studies have also found that SCCB has high-level amplifications of 1p22-32, 3q26.3, 8q24, 12q14-21, and gains of 5q, 6p, 8q, and 20q. What is more, endogens are located at some of these sites, like MDM2 [32, 36]. On the basis of the negative relationship between

p53 protein and MDM2 protein, Gupta et al. suggested that the inhibition of MDM2 ubiquitin ligase activity and the interaction of MDM2-p53 could rescue the p53 protein and achieve the purpose of inhibiting tumor [37]. Deliver the goods, there were several ongoing clinic trials of the MDM2 inhibitors in other cancers, such as acute myelocytic leukemia and myelodysplastic syndrome [38]. More interestingly, MDM2 gene amplification and TP53 gene mutation were opposed to each other in human cancer [39]. The preclinical data support this point, which was drug resistance of MDM2 inhibitors in TP53 mutant cells [40]. The mechanism of drug resistance in TP53 mutant cell is not very clear and is worth to be studied deeply.

Special-bladder molecular alterations

SCCB also harbors special-bladder molecular alterations, including TERT promoter mutation and epigenetic modifier alteration.

TERT promoter mutation

TERT promoter mutations have been found to be related to many human tumors, such as UC (55.6% and 82.8%) [41, 42]. In a retrospective study with a sample size of 11 cases, TERT promoter mutations were also high frequency in SCCB (nearly 100%), but it did not exist in small cell tumor of other organs, including lung and prostate [43]. Subsequently, by sequencing of 341 key cancer-associated genes, Chang et al. also discovered that 95% of patients with SCCB had high frequency of TERT promoter mutations, which were not found in SCLC [6]. Thus, SCCB can distinguish from small cell tumors of other organs via TERT promoter mutations.

Several studies highlighted that TERT promoter mutations were no relativity with tumor stage and grade, but were meaningful urine biomarkers for patients with papillary and flat noninvasive UC in the follow-up [44, 45]. TERT promoter mutations probably also are meaningful urine biomarker in SCCB, but hypothesis requires to be verified.

Epigenetic modifier alteration

The majority of patients with SCCB harbored a high rate of bladder-specific mutations in diverse epigenetic modifiers including ARID1A, KDM6A, CREBBP, EP300, and KMT2A/C/D [6, 7]. SCCB shared a similar frequency of these chromatin-modifying genes with UC, but not with SCLC ($P < 10^{-6}$). It suggested that SCCB was distinct from SCLC and likely came from a UC precursor [6, 8, 31]. Furthermore, these genes may be potential differential diagnosis markers in future.

The cellular origin of the SCCB

So far, the cellular origin of the SCCB is still unclear. According to histology analysis, SCCB usually mixed with other components, the most common of which is urothelium component [7]. As early as 2005, Cheng et al. presented that these two components of SCCB had common clone origin [32]. One study showed that urothelial cells could differentiate into various cell types, including glandular cells, squamous cells, and neuroendocrine cells via modulating microRNA-145 [46]. The molecular changes of SCCB are more similar to UC (discussed in 'Molecular alterations in SCCB'), which provided stronger evidence that SCCB came from a preexisting urothelial cancer. The study in preclinical model has discovered that different lesions arised from distinct precursors of UC and displayed diverse invasiveness and prognosis [47]. It is worth to discuss what kind of lesion SCCB originates from UC, because it can influence clinic treatment and management.

Treatment and follow-up

At present, there are two widely used staging systems for SCCB: The Veterans Administration Lung Study Group staging system (limited disease and extensive disease) [48] and The American Joint Committee on Cancer staging system (limited disease: T_{1-4}, N_{0-1}, M_0, and extensive disease: T_X, N_X, M_1 or T_X, N_{2-3}, M_0) [49].

Limited disease

Given the rarity of SCCB, therapeutic strategies are not well established. Over 3 decades, researchers have attempted to improve survival rate via various therapeutic strategies, including cystectomy, chemotherapy, radiotherapy, and any combination of these three treatments [50]. However, survival data are still stagnant and treatments are still based on stages and the state of patient.

Patients with limited disease (LD) can be divided into two groups: operable and bladder sparing. In 2004, a retrospective study showed that there was no survival difference between patients with cystectomy and those without cystectomy ($P = 0.65$, $N = 64$) [51]. The conclusion, which patients received initial cystectomy alone with poor survival, was consistent with some other retrospective studies [52, 53]. Therefore, researchers proposed cystectomy plus multimodal treatment (chemotherapy, radiotherapy). Some retrospective reviews suggested that patients got potential benefits of improved survival from preoperative chemotherapy [52, 54, 55]. In 2009, the results of the small phase II clinical trial are no difference with previous retrospective studies, which provided stronger evidence for the benefit of neoadjuvant chemotherapy [56]. In a retrospective analysis of 175

cohort, Lynch et al. demonstrated that besides improving long-term survival, neoadjuvant chemotherapy also could make pathological downstaging to ≤pT1N0 compared with initial cystectomy [62% vs. 9%; odds ratio: 44.55; 95% CI (10.39–191)] [57]. Thus, in 2016, The National Comprehensive Cancer Network guidelines for Oncology recommend that patients with SCCB undergo initial chemotherapy followed by radiotherapy or cystectomy without systemic disease [36].

However, the effect of adjuvant chemotherapy on survival is still not very clear in contrast with neoadjuvant chemotherapy. In some early studies, the impact of adjuvant chemotherapy on survival was not superior to cystectomy alone [52, 58]. Conversely, Kaushik et al. suggested that the 5-year overall survival was improved in patients receiving adjuvant chemotherapy, compared with those who did not (43% vs. 20%; $P=0.03$) [59]. The reasons for the difference in the effect of adjuvant chemotherapy on survival may include the following: First, these results mainly are on basis of some retrospective and single-institution studies, which have some limitations, such as small sample size, heterogeneity of chemotherapy regimen, incomparability of baseline data, and so on. Then, so far, the diagnostic criterion has not been established well and SCCB often mixes with other components. Therefore, a subset of patients only is diagnosed after cystectomy and some patients are overlooked or misdiagnosed with other bladder tumors. So, Lynch et al. promoted the effect of adjuvant chemotherapy may have been underestimated. And they suggested neoadjuvant chemotherapy was provided for SCCB diagnosed before cystectomy; however, adjuvant chemotherapy was provided when diagnosed by cystectomy [57].

In conclusion, neoadjuvant chemotherapy is necessary for patients with SCCB who can be operated on. Although the effect of adjuvant chemotherapy on survival is uncertain and need to be verified by prospective study, whether or not to use adjuvant chemotherapy depends on the situation of patient.

Patients, who have poor basic condition or are unwilling to operate, are treated with bladder-preservation therapy. In histology, SCCB was consistent with SCLC, so local radiotherapy and systemic chemotherapy was still advocated. Therefore, the bladder-preservation therapeutic strategies included TURBT plus chemoradiation or chemoradiation (sequential or concurrent chemoradiation) [50, 60, 61]. Some retrospective studies demonstrated that chemoradiation improved survival in patients with bladder sparing [50, 60–65], which is shown in Table 1. And cystectomy alone could be substituted by sequential chemoradiation, when patients aimed to preserve the bladder [50, 61, 64]. However, it is still unclear whether the curative effect of chemoradiation can be equated with that of chemotherapy plus surgery, which needs to be studied deeply.

Patients with bladder preservation have the following two problems: The optimal dose of radiation is still uncertain. In a retrospective study based on the National Cancer Database, they took 79 years old as the critical point and demonstrated that the optimal total dose between 54 and 60 Gy was recommended for patients aged 79 or younger. Conversely, among patients over the age of 79, the overall survival time was not affected by the total dose. Taking into account toxicity of radiotherapy, they also suggested that this group of patients might be better to get total dose less than 54 Gy [66]. However, this is, after all, a retrospective study, which also has many limitations as has been shown in other retrospective studies. In addition, since early retrospective studies have reported median/mean dose of radiotherapy between 59 and 64.5 Gy (Table 1), it is difficult to decide the rationality of

Table 1 Treatments, survival, and recurrence of bladder preservation

Author	Bex et al.	Bex et al.	Meijer et al.	Mattes et al.	Bryant et al.	Kamp et al.
Reference	[62]	[63]	[61]	[64]	[50]	[67]
Sample size	25	42	66	19	11	110
Stage of LD	T_XN_0-N_1M_0	$pT_XcN_{0-1}cM_0$	$T_{X-4}N_{0-1}M_0$	$T_{1-4}N_{0-2}M_0$	$T_{1-4}N_{0-2}M_0$	$cT_{1-4}N_{0-1}M_0$
LD (%)	17 (68%)	17 (40.5%)	27 (40.9%)	19 (100%)	11 (100%)	89 (80.9%)
Neoadjuvant chemotherapy	58.8%	100%	100%	89.5%	100%	73%
Local therapy TURBT	62.5%	100%	100%	100%	100%	–
Sequential radiotherapy	80%	100%	100%	100%	100%	73%
Median/mean dose of radiotherapy	60 Gy	60 Gy	60 Gy	64.8 Gy	59 Gy	60 Gy
OS	–	56% (2-year) 47% (3-year) 36% (5-year)	22% (5-year)	78% (2-year)	24% (3-year)	–
Local recurrence	12.5% (24 month)	23.5%	25.9% (29 month)	25% (24 month)	27%	22% (24 month)
Distant recurrence	–	47.1% (6 month)	44.4% (10 mo)	40% (24 month)	73% (9 month)	35% (9 month)

the choice of the critical point of age and total dose. The optimal total dose still needs further prospective studies and clinical trials to identify.

Another problem for patients receiving a bladder-conservation treatment is very little known about frequency and treatment of recurrence. The recurrence included local recurrence and distant recurrence. In some previous retrospective studies, local recurrence occurred mainly at 2 years of bladder-preservation therapy, with a recurrence rate of about 12–30% [61, 62, 64]. At the same time, the tissue components of the second primary tumor were mainly UC, carcinoma in situ (CIS), or the mixture of UC and CIS, and the small cell carcinoma (SCC) was rare [61, 63]. And the rate of response of salvage therapies, such as TURB, cystectomy, neoadjuvant chemotherapy, and BCG, was 64% [67]. Compared with local recurrence, distant recurrence first occurred 6–10 months after treatment with 30–80% recurrence frequency [50, 61, 63, 67]. The main organs involved in distant recurrence were live, lung, and bone [50]. For patients with distant recurrence, salvage therapies are still not well established. Hence, researchers promoted a reasonable follow-up schedule, which was cystoscopy and chest, abdominal/pelvic CT scanning every 3 months for the first 2 years and then 6 monthly till year 5 and then annually [67].

Extensive disease

The treatment for patients with extensive disease was similar to SCLC, which was cisplatin-based palliative systemic chemotherapy [4, 36].

Immunotherapy

Nowadays, immunotherapy is very promising therapeutic strategy for tumors, especially immune checkpoint inhibitors (ICBs). At present, the most representative ICBs are programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) inhibitor and cytotoxic T-lymphocyte protein 4 (CTLA4) inhibitor. In 2017, the US Food and Drug Administration (FDA) approved two PD-1 inhibitor (Nivolumab, Pembrolizumab) and three PD-L1 inhibitor (Atezolizumab, Durvalumab, Avelumab) for the treatment of advanced or metastatic UC [68]. SCCB shares similar molecular alterations with UC, so can ICB be used in advanced or metastatic SCCB?

In 2015, a study demonstrated that PD-L1 was absent in small cell neuroendocrine tumor cells. However, PD-L1 and PD-1 expressed in tumor-infiltrating cells (TIC), which are macrophages and lymphocytes, respectively [69]. Subsequently, in a transcriptomic and protein analysis of SCCB, Koshkin and colleagues discovered that the expression of PD-L1 was seen on TIC via immunohistochemical (IHC), but not on tumor cells [70]. Recently, the results of the study conducted by Mandelkow were concordant with Koshkin. And they promoted that SCCB exhibited an immune-excluded phenotype [71, 72]. The mechanism of the immune-excluded phenotype is not clear in SCCB. We put forward the following hypotheses: (1) The loss of RB1 and TP53 allows tumor cells getting lineage plasticity (discussed in 'Molecular alterations in SCCB'), which lets tumor camouflage to prevent TIC infiltration. (2) Although SCCB do not express PD-L1, it may release other inhibitors to keep from TIC infiltrating. To date, immunotherapy in small cell carcinoma of the bladder is not very clear; however, it also brings dawn to SCCB's novel treatment.

Conclusion and outlook

SCCB is a low incidence and high invasiveness disease. Although SCCB has small cell component, clinical manifestations and molecular alterations are similar to UC. As we all known, SCCB is difficult to distinguish from other small cell carcinomas. As we discuss in special-bladder molecular alterations, TERT promoter mutations and epigenetic modifiers highly express in SCCB, but lack in small cell carcinoma of other organs. These special mutations may be potential markers for differential diagnosis, but they need to be translated into clinically measurable indicators.

Because of the rarity of SCCB, standard treatment strategies are not well established. The treatment of SCCB mainly consists of systemic chemotherapy, surgery, and radiation, but survival data are still stagnant. Hence, we need to seek for novel therapeutic targets. Cell cycle-related therapeutic strategies may be also promising treatment options. Although immunotherapy is in full swing in treatment of tumors, in SCCB it is not optimistic. PD-L1 expresses on TIC, so does the immune checkpoint inhibitor have any effect? Indeed, tumor microenvironment and immune-excluded phenotype may be potential treatment targets.

SCCB still faces many challenges and opportunities: how to assess whether the patient with sparing bladder has a recurrence and the remedial measures after recurrence. During follow-up, are there any more sensitive serological or urine biomarkers to monitor tumor progression?

Although it is long way to improve prognosis of patients with SCCB, we believe advances in medicine will bring light.

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Compliance with ethical standards

Conflict of interests The authors have declared that no competing interest exists.

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