Genomic characterization for familial cases with urothelial carcinoma

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Abstract

In this report, we present familial cases of urothelial carcinoma. To investigate the possibility of hereditary urothelial cancer, we performed semiconductor-based next-generation DNA sequencing. A woman in her 80s who had bladder and left ureteral cancer was hospitalized in Sapporo Shirakaba-dai Hospital due to consciousness disturbance. Radiographic evaluation revealed multiple liver metastases and she died 38 days later. Needle necropsy was done for a left ureteral tumor that continued to her bladder tumor and for liver metastases. At the same time, her son in his 60s, who also had muscle-invasive bladder cancer, was admitted to Sapporo Medical University Hospital and underwent neoadjuvant chemotherapy followed by laparoscopic radical cystectomy. DNA was isolated from both cancers and normal controls in each case and analyzed by massive parallel sequencing of 409 cancer-related genes using a targeted, multiplex PCR approach followed by semiconductor sequencing. Somatic mutations of *KMT2C* and *KMT2D* were detected in the mother's tumor. Copy number gains of *FGFR1*, *IkbKB*, *NFkB2*, *FGFR2*, and *FLT3* and copy number losses of *IGF2R* and *TP53* were also found in her cancer. In her son's tumor, somatic mutations of *FGFR3* and *EP300* were identified. Copy number gains of *IkbKE/MAPK1/PARP1*, *EGFR*, *BRAF*, *IRS2*, *MAPK2K1*, *IGF1R*, and *ERBB2* and copy number loss of *TP53* were also found in his cancer. There were no germline gene mutations related to familial urothelial carcinoma. Although somatic mutation of *TP53* was a common feature, these cases with urothelial carcinoma might not be the result of a heredity syndrome.

Keywords Urothelial cancer · Familial cancer · Next-generation sequencing

Abbreviations

BCa	Bladder cancer
NMIBC	Non-muscle-invasive bladder cancer
MIBC	Muscle-invasive bladder cancer
CDDP	Cisplatin

Introduction

More than four hundred thousand bladder cancers (BCas) were newly diagnosed worldwide in 2012 [1]. Although numerous BCas are diagnosed, hereditary BCas are rarely reported. On the other hand, several hereditary diseases that may cause upper urinary tract cancer (UUTCa) have been reported. Lynch syndrome (LS) is an autosomal dominant disorder caused by germline mutations in one or more DNA mismatch repair genes that predispose patients to not only colorectal cancers but also UUTCas [2, 3]. The lifetime risk of UUTCa in patients diagnosed with LS is 14–22 times higher than in the general population [4]. The spectrum of LS is well reported in colon cancer, endometrial cancer and UUTCas. Moreover, recent studies have reported the possibility that the LS spectrum may include bladder cancer [5–7], although this remains controversial.

Costello syndrome (CS) is also known as a hereditary disease that may result in bladder cancers. CS is a rare hereditary disease, with only around 400 cases reported worldwide. In CS, there are germline mutations in the *HRAS* gene that cause several disorders, including BCas [8].

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Since general urologists encounter hereditary diseases relatively rarely, genetic examinations in familial cases are not routinely conducted. We report two familial bladder cancer cases simultaneously treated in two different hospitals for which we conducted genetic characterization to explore the possibility of inherited disease.

Case 1 (mother)

An 87-year-old woman without a history of smoking visited Sanjukai hospital presenting asymptomatic gross hematuria. Cystoscopy was performed to investigate the possibility of bladder tumors. A papillary sessile bladder tumor was found at the left ureteral orifice. In computed tomography, a left atrophic kidney with severe hydronephrosis was observed. Since the woman was elderly and had poor performance status and severe dementia, the clinician decided not to perform transurethral resection of the bladder tumor. Finally, best supportive care for the symptoms was decided. Five months after the initial hospital visit, anemia progressed and blood transfusion was performed. Approximately a year after the initial visit, the patient lost consciousness and was hospitalized in Shirakaba-dai Hospital. At that time, multiple liver metastases and a left ureteral tumor (previously observed at the left ureteral orifice) that extended to the bladder tumor were seen in CT evaluation and she finally died from urothelial cancer. Needle necropsy of the left ureteral tumor connected to the bladder tumor and liver metastases was performed. Based on hematoxylin and eosin staining, urothelial carcinoma and neuroendocrine cancer were suspected. Additional immunohistochemical staining was p63 positive and GATA3 positive. Thus, the final pathological conclusion was high-grade urothelial carcinoma in the bladder, left ureter, and liver.

Case 2 (son)

A 67-year-old man with a smoking history who had asymptomatic gross hematuria visited a general hospital and was referred to Sapporo Medical University Hospital. Cystoscopic and CT evaluations showed a 55 mm papillary sessile bladder tumor. No hydronephrosis or ureteral malignancy was observed in radiographic examinations. Because of a large intravesical tumor and severe gross hematuria, the ureteral orifices could not be detected by cystoscopy. To confirm the tumor pathology, TUR-BT was performed. Histopathological examination revealed that the tumors were high-grade urothelial carcinomas. According to preoperative enhanced MRI, the clinical T stage of the bladder cancer was cT3b, without metastases in CT evaluation. Therefore, he underwent neoadjuvant chemotherapy (gemcitabine and

cisplatin) for four cycles and received laparoscopic radical cystectomy with lymphadenectomy and construction of an ileal conduit. Three weeks after surgery, he left the hospital without any trouble. In the final pathological report, the tumor was pT1 high-grade urothelial carcinoma without lymph node metastasis (none of 65 lymph nodes). No obvious pathological effect of neoadjuvant chemotherapy was detected. The patient is alive with no evidence of disease 18 months after the radical surgery. Since we confirmed that his mother died from invasive bladder cancer, we decided to perform semiconductor-based next-generation DNA sequencing for these cases to explore the possibility of inherited bladder cancer.

Genomic DNA extraction

Paraffin blocks from the mother were cut as 8 mm sections on plain glass slides. Targeted regions for sampling were marked on adjacent hematoxylin and eosin sections by the study pathologist and recovered by scrape macrodissection. DNA was then extracted using a QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA, catalog number 56404) according to the manufacturer's instructions. DNA from frozen samples and normal blood of the son were extracted using a QIAamp DNA Mini Kit (Qiagen, catalog number 51304) following the manufacturer's protocol. Normal tissue (mother) and peripheral blood (son) were used to remove germline mutations.

Genetic testing

Barcoded libraries were generated from 40 ng of DNA per sample using the Ion AmpliSeq Comprehensive Cancer Panel and Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific, Waltham, MA, USA, catalog number 4475345) following the manufacturer's protocol. This panel contains 15,992 primer pairs in four tubes and targets almost all coding regions of 409 genes known to be related to cancer. Sequencing of templates after emulsion PCR was performed as previously described [9, 10].

Signal processing, mapping to the reference genome (hg19), and quality control were performed in Torrent Suite version 5.0 (Thermo Fisher Scientific). Somatic variants (point mutations, insertions, and deletions) were called using the Ion Reporter software 5.0 AmpliSeq Comprehensive Cancer Panel tumor-normal pair workflow (Thermo Fisher Scientific, catalog number 4477685) and default settings. A sequencing coverage of 20× and a minimum variant frequency of 10% of the total number of distinct tags were used as cutoffs. Mutations were called if they occurred in <0.1% of reads in the normal control. Germline variants were also analyzed using the Ion Reporter software 5.0

AmpliSeq Comprehensive Cancer Panel single sample workflow (Thermo Fisher Scientific). Alignment was visually inspected with Integrative Genomics Viewer software (<http://www.broadinstitute.org/igv>). Copy number variation (CNV) detection was also performed with the Ion Reporter software using an algorithm based on a hidden Markov model. Recurrent genomic regions with CNVs were identified using copy numbers greater than 3 and less than 1 for gains and losses, respectively.

Results

Somatic mutations of *KMT2C* and *KMT2D* were detected in the tumors from both the mother and son, and somatic mutations of *FGFR3* and *EP300* were identified (Table 1). Copy number gains of *FGFR1*, *IKBKB*, *NFKB2*, *FGFR2*, and *FLT3* and copy number losses of *IGF2R* and *TP53* were also observed in the mother's cancer (Fig. 1). Copy number gains of *IKBKE/MAPK1/PARP1*, *EGFR*, *BRAF*, *IRS2*,

Table 1 Somatic mutations detected in this report

Sample	Gene		Mutation type	Protein change	Coding	SIFT	PolyPhen-2	Grantham
Son	FGFR3	chr4:1806092	Missense	p.Ser371Cys	c.1111A>T	0	0.97	112
	EP300	chr22:41553299	Nonsense	p.Tyr1131Ter	c.3393_3394delTA	NA	NA	NA
	RNF213	chr17:78311659	Missense	p.Leu1565Phe	c.4695G>C	0	0.563	22
Mother	KMT2C	chr7:151945007	Missense	p.Gly838Ser	c.2512G>A	0	1	56
	KMT2D	chr12:49427282	Nonsense	p.Gln3736Ter	c.11206C>T	NA	NA	NA
	KMT2D	chr12:49445202	Frameshift insertion	p.Arg755fs	c.2263_2264insC	NA	NA	NA
	PIK3CG	chr7:106508260	Missense	p.Ala85Val	c.254C>T	0.1	0.063	64

NA not available

Fig. 1 Copy number alterations in son

MAPK2K1, *IGF1R*, and *ERBB2* and copy number loss of *TP53* were found in the son's cancer (Fig. 2). No inherited germline mutations related to familial urothelial carcinomas were observed.

Discussion

Lynch syndrome is well known to predispose patients to the risk of UUTCa, as well as colon cancer. However, whether to include bladder cancer in the LS spectrum is under discussion [11]. Since the frequency of microsatellite instability (MSI) in bladder cancer is low (3–5%) [4], the relationship between LS and bladder cancer has been considered slight. Conversely, a correlation between LS and an increased risk of bladder cancer was reported. Van der Post et al. investigated 19 LS families and found 21 bladder cancers. Moreover, 87.5% of the bladder cancer tissue was MSI positive, which indicates LS etiology [12]. Exploration of the possibility of a hereditary aspect in these urothelial cancers may benefit patients not only with regard to the screening for other malignancies but also the treatment decision. Hollande

et al. reported the benefit of adjuvant chemotherapy after radical nephroureterectomy in patients having “hereditary-like” UUTCa such as hereditary nonpolyposis colorectal cancer (HNPCC)-associated tumors [13]. Thus, investigating germline mutations in familial urothelial cancers may be an option in familial cases.

In our report, the mother (case 1) had bladder and ureteral cancer in her 80s and the son (case 2) at his 60s. Considering her age at diagnosis, it was not a typical situation for inherited cancer. However, it is of great interest that these cases had a hereditary-like aspect. Though no germline mutation was detected in these cases, somatic mutations were observed. In the son's tumor, *FGFR3* mutation was observed. Sung et al. reported the correlation of *FGFR3* somatic mutation and sensitivity to adjuvant chemotherapy [14]. In their study, for bladder cancer patients having *FGFR3* mutation, poor overall survival was confirmed among those receiving adjuvant chemotherapy. Moreover, *FGFR3* mutation could be a guide for the use of anti-*FGFR3* therapy [15]. *EP300* is one of the most mutated genes in bladder cancer [16] and there is a clinical trial evaluating the use of the histone deacetylase inhibitor mocetinostat for

Fig. 2 Copy number alterations in mother

patients having *EP300* mutation (NCT02236195). A previous study reported the potential aggressiveness of urothelial cancers having both *EP300* and *FGFR3* mutations [16]. Moreover, a case of a bladder cancer patient having *RNF213* mutation that resulted in rapid tumor growth has been reported [17]. Thus, we should be aware of possible recurrence in case 2. Moreover, in case 2, the patient had copy number gains in the *ERBB2* and *EGFR* genes. Interestingly, overexpression of these two genes has been reported to have a correlation with a higher tumor grade and recurrence rate [18]. Thus, both gene mutation and CNVs may be clinically implicated in bladder cancers. Although we could not demonstrate germline mutations in these patients, exploring gene mutations in urothelial cancers may be useful for precision medicine in the future.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Informed consent Written informed consent was obtained from the patients.

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