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Pathological and clinical insights into DICER1 hotspot mutated Sertoli-Leydig cell tumors: a comparative analysis

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Abstract

Background Sertoli-Leydig cell tumors (SLCTs) are a rare group of sex cord-stromal tumors that account for less than 0.5% of all ovarian tumors. This study aims to compare the pathological and clinical characteristics of SLCTs with and without DICER1 hotspot mutations, highlighting the impact of these genetic variations on clinical manifestation, prognosis, and pathological morphology.

Methods A retrospective analysis was conducted on 50 SLCTs. DICER1 RNase IIIb hotspot mutations were detected by the Sanger sequence. Clinical information, such as patients' symptoms, tumor staging, prognosis, and pathological features, such as tumor differentiation and growth patterns, were collected.

Results DICER1 mutation only appears in the intermediate/poorly differentiated SLCTs (35.7%), while none in the well-differentiated SLCTs. The patients with DICER1 mutation had a younger age of onset (17, 15–25) compared to the wild-type group (42, 27–58). Regarding pathological morphology, the mutant group showed a higher probability of having retiform components (40.0%) and cords or ribbon-like arrangement (33.3%). Besides, they exhibited mucinous edematous stroma (80.0%) and hemorrhage (80.0%) more frequently than the wild-type group. The mutant tumor had more mitotic figures. (11/10HPF), higher Ki-67 index (16.1%), and more CD20-positive cell infiltration. Patients of the mutant group were more likely to experience recurrence, and their tumors were more prone to rupture.

Conclusions This study demonstrates that DICER1-mutant and wildtype SLCTs have marked differences in pathological morphology and clinical manifestation. DICER1-mutatant SLCTs display worse prognosis, higher proliferative activity, and potentially more active immune microenvironments, which underscores the importance of genetic testing in diagnosing and assessing the prognosis of SLCTs.

Keywords Sertoli-Leydig cell tumors, DICER1, RNase IIIb hotspot mutations, Clinical features, Pathological characteristics

Background

Sertoli-Leydig cell tumors (SLCTs) are a rare subset of sex cord-stromal tumors, accounting for less than 0.5% of ovarian neoplasms [1]. Characterized by their varying histopathology and differentiation, these tumors often present a diagnostic challenge due to their varied clinical manifestations, ranging from virilization to non-specific abdominal symptoms. The pathological spectrum of SLCTs encompasses various histological patterns, which

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are traditionally classified based on the degree of differentiation and the presence of retiform elements [2].

Recent advances in molecular genetics have underscored the significance of DICER1 mutations in the etiology of SLCTs. The DICER1 gene, a critical component of the microRNA processing pathway, has been implicated in the tumorigenesis of various tumors, including SLCTs [3–5]. Aberrant miRNA processing resulting from DICER1 hotspot mutations is a key oncogenic event [6]. Studies have shown that SLCTs harboring DICER1 mutations tend to exhibit specific patterns in tumor behavior, histological characteristics, and clinical outcomes, distinguishing them from tumors without these mutations [4, 7, 8]. By elucidating the impact of DICER1 hotspot mutations on the clinical-pathological profile of SLCTs, researchers can pave the way for targeted therapeutic strategies and personalized patient care. This article aims to shed light on the different clinicopathological profiles between groups with and without DICER1 hotspot mutations and chart a course for future research in this pivotal area.

Methods

Patients

Ninety patients diagnosed with Sertoli-Leydig cell tumors with available formalin-fixed paraffin-embedded (FFPE) tissue in Peking Union Medical College Hospital (PUMCH) between 1994 and 2022 were identified from the medical records database. Electronic Medical Records were accessed to gather comprehensive patient data, which include age, clinical manifestations, serum examination results, menstrual history, follow-up information, and comorbidities. Tumor information was obtained from medical and pathological records, including tumor location, size, rupture, macroscopic appearance, and histopathology. After pathological examination, tumors are staged according to the FIGO (International Federation of Gynecology and Obstetrics) staging system [9]. Records of preoperative imaging examinations, intra-operative exploration, pathological examinations, and other medical files evaluated by gynecologic oncologists would be reviewed if patients received incomplete staging surgery. The study was approved by the PUMCH Institutional Review Board (JS-3230).

Sanger sequencing for detecting the DICER1 mutation

Tumor DNA was extracted from FFPE tissue according to the manufacturer's instructions. Ninety cases were amplified by polymerase chain reaction, and Sanger sequenced for DICER1 exon 24 and exon 25, where the RNase IIIb hotspot mutations lie. We designed three pairs of PCR primers targeting these regions (pair 1, forward:5′-CTTTTCTGCAATCAAAATGAAAGA ATAAT-3′; reverse:5′-CTGTGGACTGCCTG TAAAAG TGG-3′; pair 2, forward: 5′-CTTTAGACCACTATG CCGTCAGAAC-3′; reverse: 5′-CTCATAACCAAG CACCTTTATGAAGAC-3′; pair 3, forward: 5′-CGT ACTTTACAGCCAGCGATGC-3′; reverse: 5′-TTCTTC GGATTTGGGGATCAG-3′). Variants of nucleotide and amino acid numbering were annotated based on the DICER1 reference sequence (GenBank: NM_177438).

Six samples' tissue was degraded, and 34 samples partially or wholly failed the sequencing. Ultimately, 50 samples successfully yielded DICER 1 hotspot mutation profiles and were included in the final data analysis.

Histopathological and immunohistochemical evaluation of primary tumors

All primary surgical specimens underwent pathological examination, and the diagnosis was made based on the WHO Classification of Tumours Editorial Board. Female genital tumours [Internet] [1]. Two pathologists independently reviewed all cases, and a third pathologist arbitrated disagreements to reach a consensus.

Specific arrangement patterns were deemed present if they comprised ≥ 10% (50% for solid patterns) of a single section. Mucinous edematous stroma was considered present if it comprised ≥ 5% of a single section, and hemorrhage was deemed present if it comprised ≥ 1%. Any additional observed pathological characteristics were recorded. Cells in 10 random high-power fields (400x) were counted to determine the mean mitosis rate. The nuclear-to-cytoplasmic (N/C) ratio was calculated using the digital pathology software QuPath [10].

Immunohistochemistry was performed on 3 μm FFPE tissue sections using antibodies for Ki-67, CD3, CD20, and PD-L1. The Ki-67 index and N/C ratio were calculated using the digital pathology software QuPath [10]. Cells in 5 random fields (at least 5000 cells in each field) were counted to determine the mean Ki-67 index and the N/C ratio. CD3 and CD20 cells were counted manually on representative sections by immunohistochemical staining of all cases. Intratumoral ≥ 20 sparse or focal CD3 positive cells were interpreted as CD3 positive, and ≥ 1 cluster of CD20 positive cells (≥ 20 for one cluster) within tumor was interpreted as CD20-positive. PD-L1 scores were far below 1 for Combined Positive Score (CPS) and 1% for Tumor cell Proportion Score (TPS). Integrating PD-L1's actual staining pattern in our study and to investigate potential intergroup disparities, PD-L1 positivity was defined as ≥ 10 contiguous tumor cells exhibiting complete circumferential membranous staining.

Statistical analysis

The data distribution comparison was conducted using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA) and further processed using GraphPad Prism 8 software (San Diego, CA, USA). Continuous and categorical variables were analyzed using the chi-square or Fisher's exact test. *P*-values and 95% confidence intervals (CIs) were calculated.

Results

Cohort basic information of SLCTs

Among the 50 patients (Table 1), diagnostic age ranged from 12 to 73 years, with a mean age of 38, predominantly of reproductive age(58%). There was no significant difference in the tumor location between the right and left ovary (48% vs. 50%). Clinical symptoms were primarily characterized by non-specific abdominal masses (78%), with approximately half of the patients (48%) exhibiting androgen-related symptoms and 11 patients (22%) presenting with abnormal vaginal bleeding.

According to the fifth edition of the WHO grading criteria [1], the cases were categorized as follows: 22 cases of poorly differentiated tumors (44%), 20 cases of intermediate differentiated tumors (40%), and 8 cases of well-differentiated tumors (16%). According to the FIGO staging system (2014 version) [9], all 50 patients were classified as FIGO stage I, with 39 cases (78%) in FIGO IA, 1 case (2%) in FIGO IB, and 10 cases (20%) in FIGO IC.

All patients underwent follow-up by outpatient records or telephone; four patients were lost to follow-up. The median follow-up time was 66.5 months. Three patients (6%) experienced tumor recurrence after surgery, with one patient experiencing two recurrences, and no patients died from SLCTs during the follow-up period. Additionally, one patient died of heart disease 159 months after surgery (at age 87, SLCT diagnosed at age 73).

DICER1 RNase IIIb hotspot mutations of SLCTs

Fifteen of 50 patients carried DICER1 RNase IIIb hotspot mutations, which exhibited specific diversity. The mutations carried by 14 patients had been reported previously and were all somatic missense mutations [5, 11–16]. The most common mutation in this cohort was c.5125G > A (p.Asp1709Asn), detected in four patients, further demonstrating this as a common mutation site based on prior literature reports [12, 17]. The second most common mutations were c.5126A > G (p.Asp1709Gly) and c.5437G > C (p.Glu1813Gln) in 2 patients, respectively. Additionally, an unreported DICER1 RNase IIIb nucleotide mutation was identified in our cohort, which is c.5150delC (p.Lys1718Serfs*15), indicating a deletion of cytosine at position 5150, resulting in a frameshift that leads to premature termination of the transcript at exon 24. The clinical significance of this mutation remains unclear. This patient was followed for 77 months without recurrence or metastasis after undergoing laparoscopic adnexectomy.

The basic clinical and pathological information of 15 patients carrying hotspot mutations is provided in Table 2. The remaining 35 patients did not carry DICER1 RNase IIIb hotspot mutations, including 8 patients diagnosed as well-differentiated SLCTs.

Comparison of clinicopathological features in well differentiated and intermediate/poorly differentiated SLCTs

This cohort included 8 cases of well-differentiated SLCTs (16%) and 42 cases of intermediate/poorly differentiated SLCTs (84%). The sequencing results of this cohort showed that mutations in the DICER1 RNase IIIb hotspot region were only present in intermediate/poorly differentiated SLCTs, not in well-differentiated SLCTs.

The age of onset in the well-differentiated group was generally older than that in the intermediate/poorly differentiated group (median age 51.5 vs. 32), with 5 cases of 8 in the postmenopausal stage. In contrast, the intermediate/poorly differentiated group primarily consisted of patients of childbearing age [18, 19]. In terms of clinical symptoms, the well-differentiated group exhibited more frequent abnormal vaginal bleeding, which showed statistical significance between the two groups ($P=0.039$).

According to the FIGO staging system (2014 version) [9], all patients with well-differentiated tumors were classified as FIGO IA and IB, while 10 cases (23.8%) in the intermediate/poorly differentiated group were classified as FIGO IC. In this study, all recurrence cases occurred in the intermediate/poorly differentiated group (3 cases, 6%), while none of the 8 patients in the well-differentiated group experienced tumor recurrence or metastasis during follow-up.

By further comparing the pathological features of the two groups, the primary tumors in the well-differentiated SLCT group and the intermediate/poorly differentiated SLCT group were similarly distributed in the left and right ovaries. In the well-differentiated group, there was 1 case with bilateral synchronous primary SLCTs. The maximum diameter, N/C ratio, mitotic count, and Ki-67 index of tumors in the intermediate/poorly differentiated group were statistically significantly higher than those in the well-differentiated group (*P* values were 0.002, 0.002, 0.003, and 0.004, respectively). Additionally, 28 cases (56%) of tumors in the intermediate/poorly differentiated group exhibited solid components, significantly easier to scope than in the well-differentiated group ($P=0.001$).

Table 1 Clinicopathological features of the entire SLCT cohort ($n=50$)

	Well differentiated	**Moderately/Poorly differentiated**				**Total**	**Difference between groups**
	***N*=8(16.0%)**	***Mutants***	***Wild-type***	***Difference between groups***	**Total**	***N*=50**	***P***
		N=15(35.7%)	***N=27(64.3%)***	***P***	***N*=42(84.0%)**		
Age, M(P_{25}, P_{75})	51.5(28.5, 56.0)	*17.0(15.0,25.0)*	*42.0(27.0,58.0)*	***<0.001***	32.0(18.5, 54.5)	33.5(20.5, 54.5)	0.165
Age phase, n (%)				***0.001***			0.081
Pre-childbearing age	0	*4(26.7%)*	*0*		3(7.1%)	3(6.0%)	
Childbearing age*	3(37.5%)	*10(66.7%)*	*15(55.6%)*		26(61.9%)	29(58.0%)	
Postmenopausal	5(62.5%)	*1(6.7%)*	*12(44.4%)*		13(31.0%)	18(36.0%)	
Symptom, n (%)							
Virilization	3(37.5%)	*7(46.7%)*	*14(51.9%)*	*0.750*	21(50.0%)	24(48.0%)	0.521
Abnormal vaginal bleeding	4(50.0%)	*2(13.3%)*	*5(18.5%)*	*0.669*	7(16.7%)	11(22.0%)	***0.039***
Abdominal mass	7(87.5%)	*13(86.7%)*	*19(70.4%)*	*0.240*	32(76.2%)	39(78.0%)	0.484
FIGO stage				***0.010***			0.377
IA	7(87.5%)	*8(53.3%)*	*24(88.9%)*		32(76.2%)	39(78.0%)	
IB	1(12.5%)	*0*	*0*		0	1(2.0%)	
IC	0	*7(46.7%)*	*3(11.1%)*		10(23.8%)	10(20.0%)	
Tumor markers							
AFP ng/ml	3.1(1.5, 12.8)	*9.5(3.6, 87.4)*	*3.4(2.2, 10.4)*	*0.103*	5.3(2.5, 21.3)	4.9(2.3, 20.6)	0.208
CA125 U/ml	9.9(7.6, 13.2)	*16.7(10.4, 46.6)*	*12.3(8.9, 22.8)*	*0.276*	14.3(9.6, 25.1)	12.7(9.2, 22.8)	0.163
CA199 U/ml	14.0(5.8, 24.1)	*8.3(5.2, 20.3)*	*9.5(6.5, 18.1)*	*0.282*	9.5(5.8, 18.1)	9.5(5.8, 19.7)	0.564
CEA ng/ml	2.5(1.2, 4.0)	*0.8(0.6, 1.3)*	*1.4(0.9, 1.8)*	*0.054*	1.2(0.7, 1.6)	1.3(0.8, 1.8)	***0.041***
Recurrence, n(%)				***0.017***			0.440
None	8(100%)	*12(80.0%)*	*27(100%)*		39(92.9%)	47(94.0%)	
Once	0	*2(13.3%)*	*0*		2(4.8%)	2(4.0%)	
Twice	0	*1(6.7%)*	*0*		1(2.4%)	1(2.0%)	
Follow-up, month	39.5(22.0, 86.3)	*76.0(48.0,95.0)*	*67.0(28.0,120.0)*	*0.969*	72.5(40.8, 115.3)	66.5(35.8, 114.3)	0.218
Tumor site, n(%)				*0.237*			0.832
Left ovary	4(50.0%)	*9(60.0%)*	*11(40.7%)*		20(47.6%)	24(48.0%)	
Right ovary	3(37.5%)	*6(40.0%)*	*16(59.3%)*		22(52.4%)	25(50.0%)	
Both ovaries (synchronous)	1(12.5%)	*0*	*0*		0	1(2.0%)	
Maximum diameter (cm)	3.1(2.6, 4.3)	*6.0(5.0,10.5)*	*6.0(3.3,8.0)*	*0.117*	6.0(4.2, 9.0)	5.1(3.3, 8.3)	***0.002***
Differentiation, n (%)				*0.927*			
Well	8(100%)	*0*	*0*		0	8(16.0%)	
Intermediate	0	*7(46.7%)*	*13(48.1%)*		20(47.6%)	20(40.0%)	
Poorly	0	*8(53.3%)*	*14(51.9%)*		22(52.4%)	22(44.0%)	
DICER1 hotspot mutation	0	*15(100%)*	*0*		15(35.7%)	15(30.0%)	***0.046***
Histopathology, n (%)							
Retiform differentiation	0	*6(40.0%)*	*0*	***<0.001***	6(14.3)	6(12.0%)	0.259

Table 1 (continued)

	Well differentiated	Moderately/Poorly differentiated				Total	Difference between groups
	N = 8(16.0%)	*Mutants*	*Wild-type*	*Difference between groups*	Total	*N* = 50	*P*
		N = 15(35.7%)	*N* = 27(64.3%)	*P*	*N* = 42(84.0%)		
Heterologous elements	0	*1(6.7%)*	*0*	*0.180*	1(2.4%)	1(2.0%)	0.663
Solid	0	*10(66.7%)*	*18(66.7%)*	*1.000*	28(66.7%)	28(56.0%)	***0.001***
Tubule	7(87.5%)	*10(66.7%)*	*11(40.7%)*	*0.112*	21(50.0%)	28(56.0%)	0.053
Nests	5(62.5%)	*10(66.7%)*	*18(66.7%)*	*1.000*	28(66.7%)	33(66.0%)	0.821
Cords or ribbon-like	0	*5(33.3%)*	*2(7.4%)*	***0.033***	7(16.7%)	7(14.0%)	0.218
Background							
mucinous edematous stroma	3(37.5%)	*12(80%)*	*10(37.0%)*	***0.008***	21(50%)	25(50.0%)	0.445
hemorrhage	2(25%)	*12(80%)*	*9(33.3%)*	***0.004***	21(50%)	23(46.0%)	0.198
Inflammatory cells							
CD3	7(87.5%)	*14(93.3%)*	*19(70.4%)*	*0.086*	33(78.6%)	40(80.0%)	0.567
CD20	2(25%)	*10(66.7%)*	*5(18.5%)*	***0.002***	15(35.7%)	17(34.0%)	0.562
PD-L1	2(25%)	*8(53.3%)*	*14(51.9%)*	*0.927*	22(52.4%)	24(48.0%)	0.160
N/C ratio	0.3109(0.2868, 0.3286)	*0.3394(0.3320,0.3476)*	*0.3376(0.3298,0.3528)*	*0.834*	0.3385(0.3298, 0.3504)	0.3364(0.3241, 0.3491)	***0.002***
Mitotic figures(/10HPF)	0(0, 8)	*11(9,17)*	*6(1,12)*	***0.003***	9(2, 13)	8(0.8, 12.3)	***0.003***
Ki-67(%)	3.7(1.2, 6.1)	*16.08(12.56,28.48)*	*6.90(3.89,12.31)*	***< 0.001***	10.4(4.6, 17.5)	9.7(4.0, 14.4)	***0.004***

* Childbearing age: 16 years old to menopause [18, 19]

Table 2 The clinicopathological and genetic information of 15 cases with DICER1 RNase IIIb hotspot mutations

Age at diagnosis	Tumour site	FIGO	Differentiation	Exon	Variation	Oncologic outcomes (follow-up time, months)
25	right	IC	intermediate	24	c.5125G > A (p.Asp1709Asn)	NED (1)
19	right	IA	poorly	25	c.5437G > C (p.Glu1813Gln)	NED (124)
14	left	IA	intermediate	25	c.5425G > A (p.Gly1809Arg)	NED (119)
72	right	IA	poorly	25	c.5504A > C (p.Tyr1835Ser)	NED (115)
16	right	IC	intermediate	24	c.5125G > A (p.Asp1709Asn)	NED (95)
12	left	IC	poorly	24	c.5125G > A (p.Asp1709Asn)	NED (80)
17	left	IC	poorly	25	c.5439G > C (p.Glu1813Asp)	First relapse(right ovary, focal; DFS, 14), tumor section, second relapse(abdominal and pelvic cavities; DFS,38), tumor section, NED (24)
21	left	IA	poorly	24	c.5150delC(p.Lys1718Serfs*15)	NED(77)
17	right	IA	intermediate	25	c.5428G > C (p.Asp1810His)	Relapse (right ovary; DFS, 48), tumor section, NED (28)
16	left	IC	poorly	24	c.5126A > G (p.Asp1709Gly)	NED(66)
17	right	IC	intermediate	24	c.5126A > T (p.Asp1709Val)	NED(73)
15	left	IC	intermediate	25	c.5429A > T (p.Asp1810Val)	Relapse (left ovary; DFS, 13), tumor section, NED (40)
34	left	IC	intermediate	25	c.5437G > A (p.Glu1813Lys)	NED(48)
12	left	IA	poorly	24	c.5125G > A (p.Asp1709Asn)	NED(45)
40	left	IA	poorly	24	c.5127 T > A (p.Asp1709Glu)	NED(43)

Abbreviations: FIGO International Federation of Gynecology and Obstetrics, *DFS,n* disease-free survival (relapse at n months at follow-up after diagnosis or previous relapse), *NED* no evidence of disease (after diagnosis or relapse)

Comparison of clinicopathological features in intermediate/poorly differentiated SLCTs with hotspot mutations and wild-type counterparts

Forty-two cases of intermediate/poorly differentiated SLCTs were analyzed and categorized into two groups based on the presence of DICER1 RNase IIIb hotspot mutations: the DICER1 RNase IIIb hotspot mutant group includes 15 cases (35.7%), and the group without DICER1 RNase IIIb hotspot mutations (referred to as wild-type) consists of 27 cases (64.3%).

Significant age differences were observed between patients with DICER1 mutations and those without. The median age of patients with DICER1 mutations was 17 years (P_{25}-P_{75}: 15–25), while the wild-type group had a median age of 42 years (P_{25}-P_{75}: 27–58), indicating that the DICER1 mutant group leans toward early onset ($p < 0.001$). Analysis based on life stages revealed that the mutant group had a higher incidence of tumors during the childbearing age (73.3%) compared to 55.6% in the wild-type group. Conversely, the incidence during the postmenopausal period was higher in the wild-type group (44.4%), while the mutant group had only 6.7%.

Clinical symptoms were generally similar between the two groups, with 46.7% of patients in the mutant group exhibiting androgen-related symptoms, compared to 51.9% in the wild-type group. The occurrences of abnormal vaginal bleeding and abdominal masses were similar in both groups.

According to the FIGO staging system (2014 version) [9], all tumors in the cohort were classified as stage IA or IC. However, the mutant group had 7 cases (46.7%) with tumor rupture, which was statistically significant compared to 3 cases (11.1%) in the wild-type group. All patients were followed up, except 1 case lost to follow-up in the mutant group and 3 in the wild-type group. The follow-up duration between the two groups did not show statistical significance. The recurrence rates were markedly different between the two groups, with a recurrence rate of 20% (3 cases of 15) in the mutant group and no recurrence in the wild-type group.

The growth patterns between the two groups were significantly different. The mutant group was more likely to exhibit reticular components (40.0% vs. 0%) (Fig. 1A and 1E) and displayed cords or ribbon-like patterns (33.3% vs. 7.4%) (Fig. 1B and 1F), highlighting its unique morphological features associated with DICER1 hotspot mutations.

The tumor's microenvironment in the mutant group showed a higher incidence of mucinous edematous backgrounds (80%) (Fig. 1C) and hemorrhage (80%) (Fig. 1D), while the wild-type group did not (Fig. 1G and 1H). Inflammatory cells, particularly CD20-positive cells, predominantly distributed in clusters around small blood vessels within the tumor, were also more prevalent in the mutant tumors (66.7%) (Fig. 2C). Additionally, there was one case of a patient with a DICER1 RNase IIIb hotspot mutation, in which the tumor tissue exhibited the formation of tertiary lymphoid structures.

The Ki-67 proliferation index and mitotic figures in the mutant group (Fig. 2A) are higher than those in the wild-type group (Fig. 2B), with a median Ki-67 index of 16.08% in the mutant group compared to 6.90% in the wild-type group and median mitotic figures of 11 in the mutant group compared to 6 in the wild-type group.

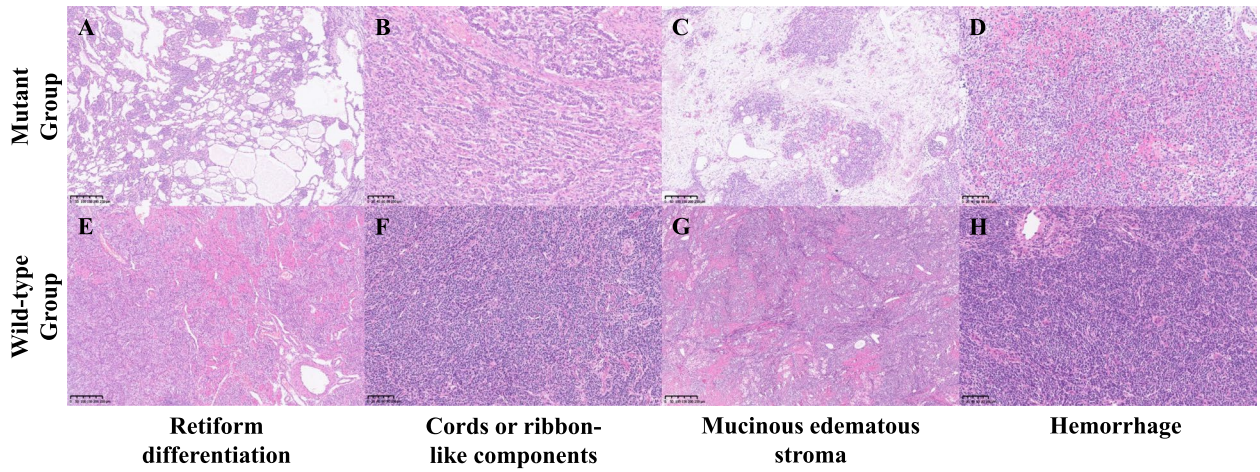

Fig. 1 Morphological comparison between mutant group and wild-type group. Representative images of Retiform differentiation (**A**, **E**), Cords or ribbon-like (**B**, **F**), Mucinous edematous stroma (**C**, **G**) and Hemorrhage (**D**, **H**). Mutant group exhibits more retiform differentiation (**A**), cords or ribbon-like arrangement (**B**), obvious mucinous edematous stroma (**C**) and hemorrhage (**D**). In contrast, wild-type group rarely exhibits retiform differentiation (**E**), cords or ribbon-like arrangement (**F**), mucinous edematous stroma (**G**) and hemorrhage (**H**)

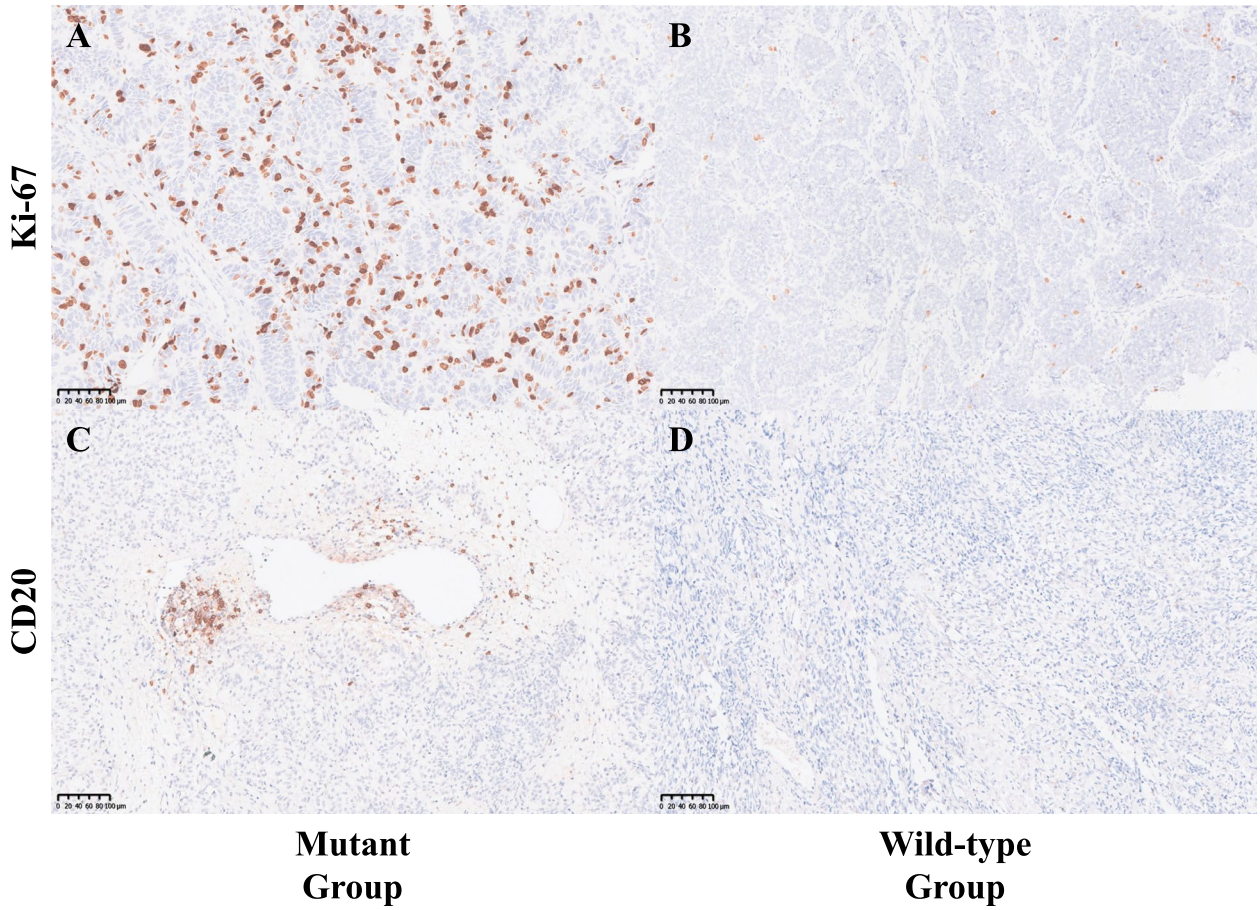

Fig. 2 Comparison of Ki-67 and CD20 expression between mutant group and wild-type group. Representative images of Ki-67 (**A**, **B**) and CD20 (**C**, **D**). **A**, Mutant group exhibits higher Ki-67 index; **B**, Wild-type group has a lower Ki-67 index; **C**, Mutant group has more CD20 positive cells in the stroma; **D** Wild-type group barely has CD20 positive cells in the stroma. (Magnification 200x)

The median follow-up time for the 42 intermediate/poorly differentiated SLCT patients was 72.5 months (range: 40.75 to 115.25 months). There were no deaths by the end of the study, resulting in a 100% survival rate related to SLCTs. Three patients experienced recurrence, all of which were in the mutant group. Kaplan–Meier survival analysis suggested a statistically significant difference in progression-free survival (PFS) between the mutant group and the wild-type group ($P=0.0236$) (Fig. 3).

Discussion

It was first proposed in 2017 that well-differentiated SLCTs differ from intermediate or poorly differentiated ones in terms of their pathogenesis [4]. Since then, an increasing number of scholars have agreed that well-differentiated SLCTs and intermediate to poorly differentiated SLCTs belong to two entities of different origins. Our findings further support that well-differentiated SLCTs and intermediate/poorly differentiated SLCTs are two distinct tumor entities. There are significant differences between the two regarding gene mutations and pathological morphology.

Additionally, we revealed significant differences between the mutant and wild-type groups of intermediate/poorly differentiated SLCTs regarding clinical features, tumor characteristics, and outcomes. Specifically, the younger median age at onset in the mutant group is consistent with previous literature [8], suggesting that DICER1 mutations are associated with a tendency for early-onset tumors. Heravi-Moussavi et al. [5] reported a significant association between DICER1 mutations and early-onset SLCTs, emphasizing the genetic component of the disease.

The clinical manifestations observed in our cohort, including androgen-related symptoms and abdominal masses, are consistent with the clinical spectrum of SLCTs. However, we found no statistically significant differences in these symptoms between the DICER1 mutant group and the wild-type group, which may provide new

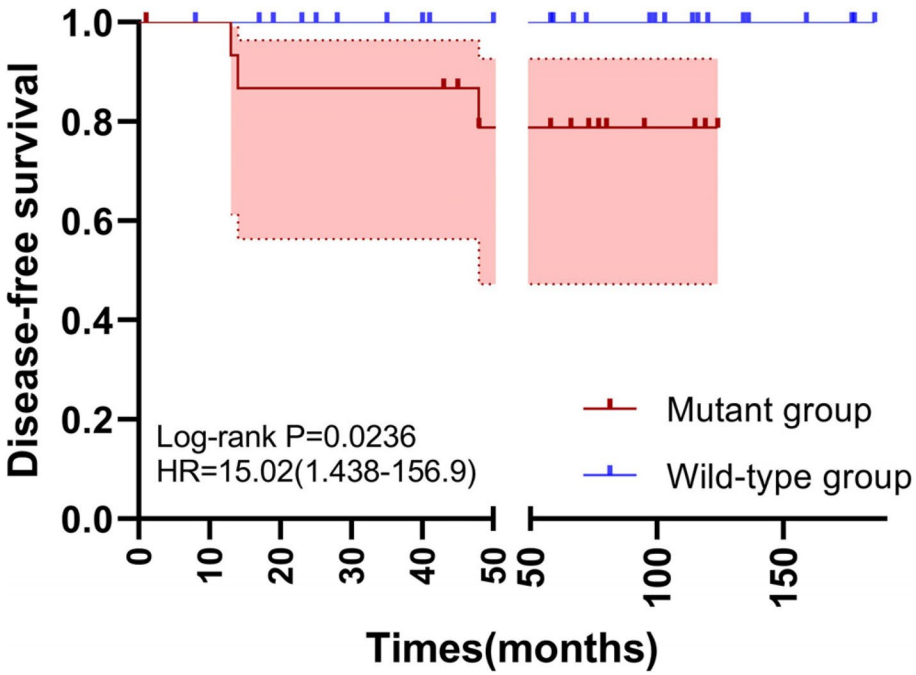

Fig. 3 Survival analysis comparison between mutant group and wild-type group. Sertoli-Leydig cell tumor disease-free survival of mutant group (red) and wild-type group (blue) with 95% confidence interval shading included

insights into the heterogeneity of SLCTs. This observation differs from the classic description by Young and Scully [8], suggesting a more complex interaction between genetics and clinical manifestations, which needs a more comprehensive cohort to elucidate.

Based on the mutations in the DICER1 RNase IIIb hotspot region, we conducted a detailed analysis of tumor characteristics and differentiation, leading to new findings: the mutant group has particular pathological morphology, more mitotic figures, and higher Ki-67 index, indicating more active proliferative activity in the mutant group and a potential link between DICER1 mutations and the dynamics of tumor growth. Furthermore, immunohistochemical staining for CD20 reveals more B cell infiltration around small blood vessels within the tumor in mutant group, suggesting a potentially more active immune response and microenvironment. All of these parameters provide new insights into understanding the tumor behavior. These findings further enrich the research by Schultz et al. [20], which highlighted the morphological diversity of DICER1-associated tumors and suggested that these mutations contribute to specific histopathological characteristics.

Regarding clinical outcomes and recurrence rates, our study explored the impact of DICER1 mutations on the prognosis of SLCTs. Patients with the mutation are more prone to recurrence, indicating that patients with DICER1 mutant SLCTs may require more stringent follow-up, as DICER1 RNase IIIb hotspot mutations might be associated with poor prognosis. Our finding also suggests that tumors with mutations are more likely to rupture than wild-type tumors. Though the higher recurrence rate in the mutant group in poorly/intermediate differentiated SLCTs is consistent with the previous study on SLCTs [2, 21, 22]. Previous studies have not described the corresponding DICER1 mutation status, and our study found that the relapsed cases were all DICER1 mutation cases, suggesting the need to detect DICER1 status.

In our cohort, the clinical outcomes of patients with DICER1 mutations ranged from complete remission to recurrence, highlighting the prognostic uncertainty associated with these mutations. Whether specific DICER1 mutations were associated with higher risks of recurrence or whether other interacting factors, for example, tumor microenvironment, the patient's immune response, or additional genetic alterations, may influence tumor behavior and treatment response, would be a potential investigation direction. Given the small size of our study cohort, further large-scale studies are necessary to more comprehensively understand the role of DICER1 mutations in the pathogenesis and progression of SLCTs. Specifically, exploring the effects of different DICER1 mutations on the miRNA processing pathway and identifying downstream targets affected by these mutations could provide potential new therapeutic

targets. Moreover, longitudinal studies on the clinical outcomes of SLCT patients carrying DICER1 mutations in larger cohorts will be crucial for developing more effective monitoring strategies and personalized treatment approaches.

Conclusion

Our study discovered that DICER1 mutant type and wild-type SLCTs have distinct growth patterns and stromal backgrounds in pathological morphology, expanding the understanding of SLCTs. And recurrences in our cohort occurred only in the mutant group of intermediate/poorly differentiated SLCTs. It highlights the key role of DICER1 in the clinical progression of intermediate/poorly differentiated SLCTs and in predicting disease prognosis. Due to distinct clinical outcomes, genetic testing is essential for diagnosing and predicting prognosis in SLCTs.

Abbreviations

FFPE	Formalin-fixed paraffin-embedded
FIGO	International Federation of Gynecology and Obstetrics
N/C ratio	Nuclear-to-cytoplasmic ratio
SLCT	Sertoli-Leydig cell tumors

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Authors' contributions

•S.Y. and J. C. conceived and designed the manuscript; •ZY. L. and Y. L. collected the data; •ZY. L., P. W., and S. M. performed the experiment; •ZY. L., ZH. L., and J. C. performed the pathology investigation; •ZY. L., JC. C., and XL. C. performed the data analysis; •ZY. L. wrote the manuscript; •S.Y., J. C., XY. C., and H.M. made critical revisions to the manuscript; •S.Y., ZH. L., and J. C. contributed to project administration and funding acquisition.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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