DICER1 and *FOXL2* Mutation Status Correlates With Clinicopathologic Features in Ovarian Sertoli-Leydig Cell Tumors

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Sertoli-Leydig细胞瘤

- **定义：**肿瘤由不同比例的Sertoli 细胞和Leydig 细胞组成，在中分化和低分化肿瘤中可能有原始的性腺间质成分，有时伴有异源性成分。
- **ICD-O 编码**

Sertoli-Leydig 细胞瘤，高分化　8631/0

Sertoli-Leydig 细胞瘤，中分化　8631/1

Sertoli-Leydig 细胞瘤，中分化伴异源性成分　8634/1

Sertoli-Leydig 细胞瘤，低分化　8631/3

Sertoli-Leydig 细胞瘤，低分化伴异源性成分　8634/3

Sertoli-Leydig 细胞瘤，网状型　8633/1

Sertoli-Leydig 细胞瘤，网状型，伴异源性成分　8634/1

Sertoli-Leydig细胞瘤

- **流行病学**：Sertoli-Leydig 细胞瘤罕见，占卵巢肿瘤的0.5%以下；中分化和低分化类型最常见。据报道，Sertoli-Leydig细胞瘤发生于1-84 岁女性，平均年龄25 岁。伴有DICER-1 种系突变的肿瘤，中位年龄13 岁。伴有明显的网状结构的肿瘤也发生于较年轻患者，中位年龄15 岁。
- **临床特征**：40%～60%患者出现男性化表现，偶尔患者呈雌激素表现。雄激素表现包括闭经、多毛症、乳房萎缩、阴蒂肥大和声嘶，而雌激素影响包括同性假性早熟和子宫不规则出血。患者可能表现为腹部疼痛、腹水或肿瘤破裂。就诊时大约2-3%的肿瘤有卵巢外播散，但是淋巴结转移罕见。
- 大体检查：超过97%的Sertoli-Leydig 细胞瘤是单侧性。大小范围2-35cm（平均12-14cm）。它们可能是实性、囊实性或罕见囊性。实性区域呈肉样、淡黄色、暗红色或灰色。偶见出血坏死区域，也可能发生扭转和梗死。

Sertoli-Leydig细胞瘤

- 根据Sertoli 细胞成分呈管状分化的程度（随级别升高而减少）和原始性腺间质的数量（随级别升高而增多），将Sertoli-Leydig 细胞瘤进一步分类为高分化、中分化和低分化亚型。
- Leydig 细胞随着级别升高而减少。
- 异源性成分和/或网状结构可见于中分化和低分化亚型。

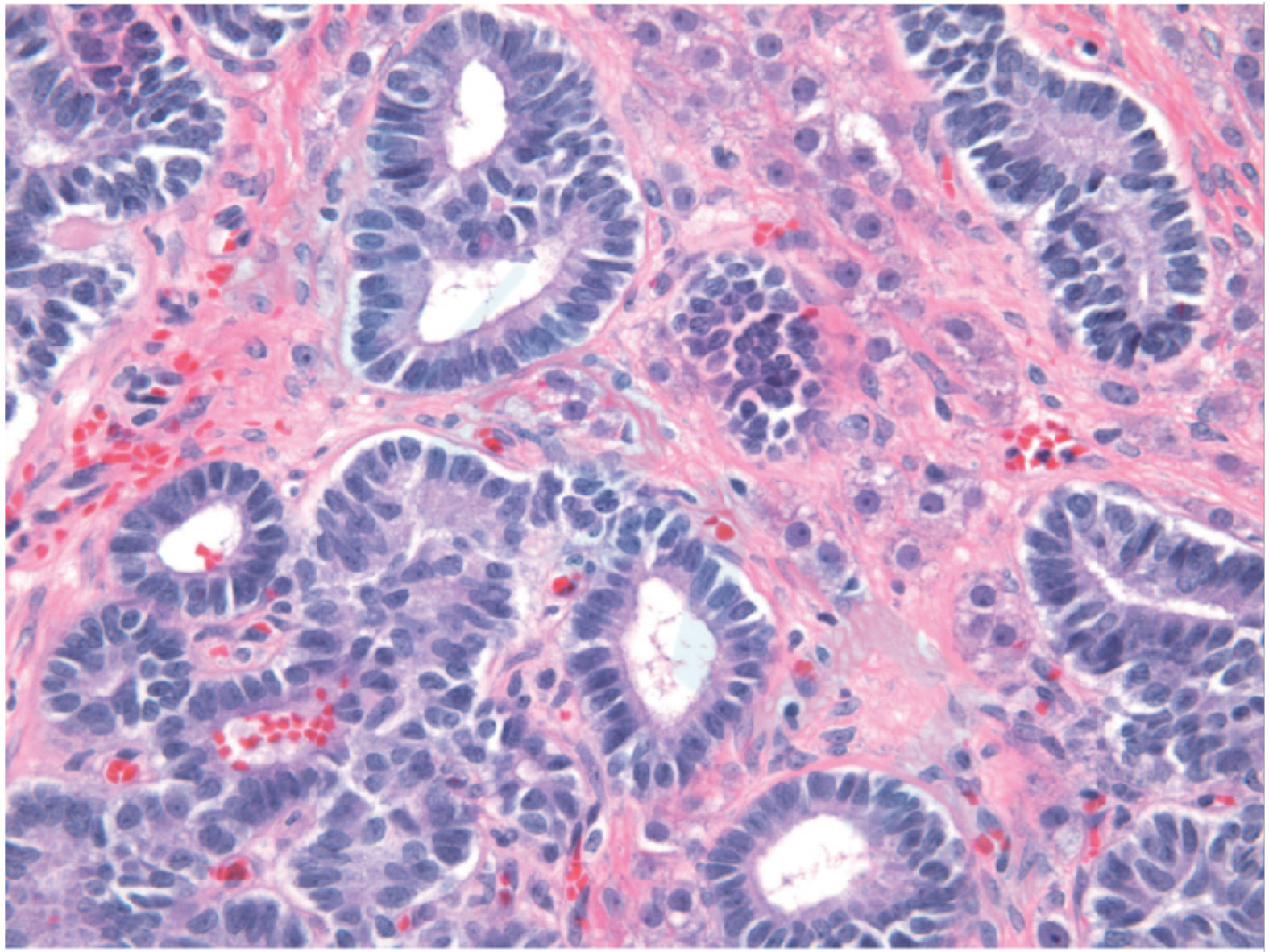
Sertoli-Leydig细胞瘤

组织病理学

•在高分化Sertoli-Leydig 细胞瘤中，Sertoli 细胞形成中空或实性小管，缺乏显著的核异型性或核分裂活性。有纤细的纤维性间质，间质中有小管状、条索状或单个散在的Leydig 细胞。

•在中分化肿瘤中，深染的Sertoli 细胞形成细胞丰富的小叶状结构，胞质稀少，杂乱地混合着Leydig 细胞，通常由水肿性间质分隔。某些病例可出现巢状至腺泡状排列的Sertoli 细胞，肿瘤的整体印象可能因具有被覆Sertoli 细胞的中空和实性小管而夸大。Sertoli 细胞通常仅有轻度细胞异型性，但可能偶见奇异形退变性核异型性，并非预后不佳的表现。

•低分化肿瘤以类似于原始性腺间质的肉瘤样间质为主要特征，通常只有很少程度的中分化肿瘤样分叶状排列，以致仅凭肿瘤的大多数区域无法诊断为Sertoli-Leydig 细胞瘤。低分化肿瘤的核分裂率不一，许多区域核分裂率高，通常高达20/10HPF。



Well-differentiated Sertoli–Leydig cell tumor. Well-formed Sertoli tubules are intermixed with Leydig cells.

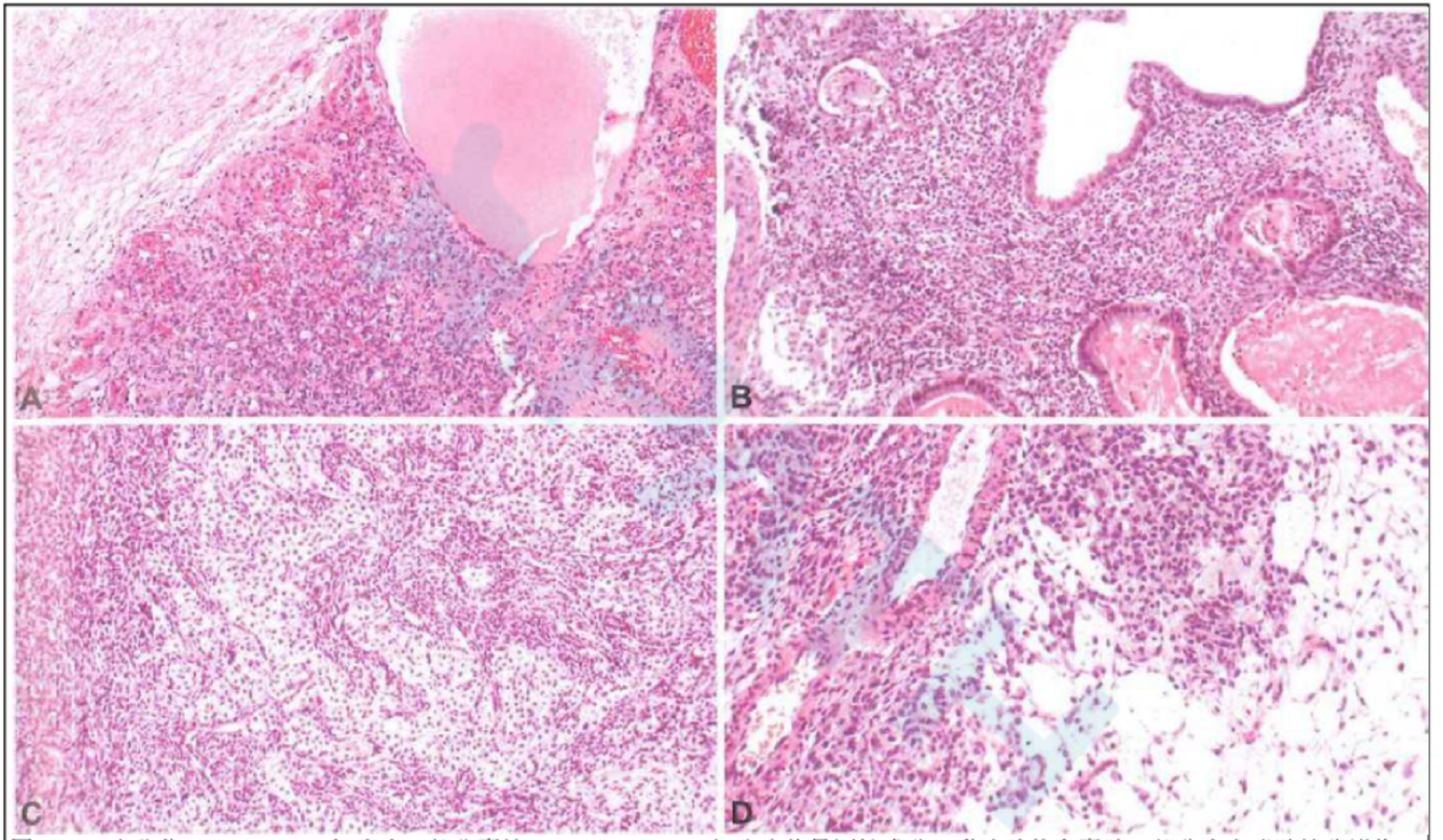
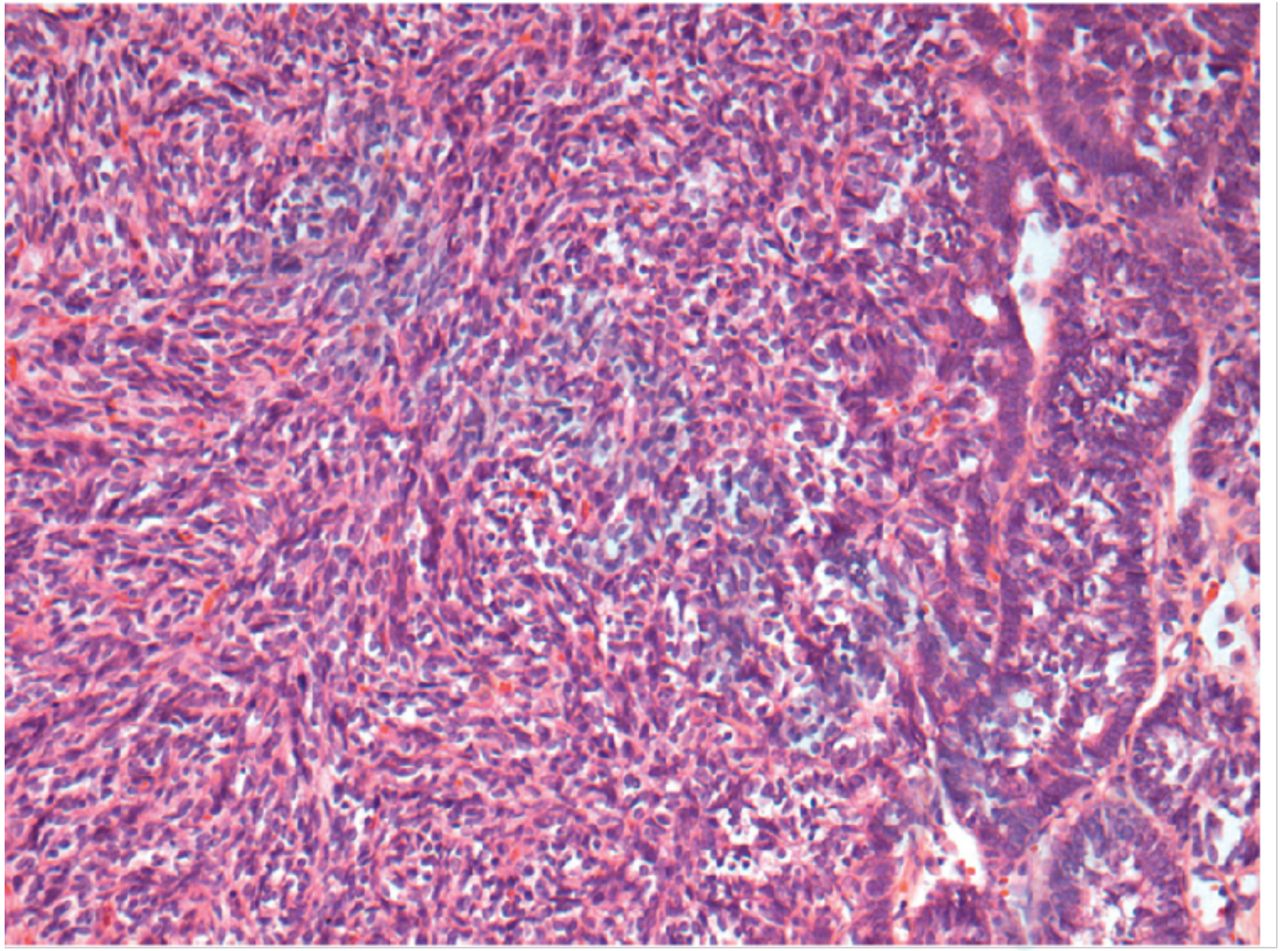


图 1.51 A 中分化 Sertoli-Leydig 细胞瘤，部分囊性。B Sertoli-Leydig 细胞瘤伴异源性成分。黏液腺体和囊肿，部分含有嗜酸性分泌物，陷于其他方面典型的 Sertoli-Leydig 肿瘤背景中。C 中分化 Sertoli-Leydig 细胞瘤。肿瘤主要为 Sertoli 细胞，呈巢状至实性管状。D 中分化 Sertoli-Leydig 细胞瘤。少细胞区和多细胞区，后者显示深染的 Sertoli 细胞掺杂着 Leydig 细胞。



Poorly differentiated Sertoli-Leydig cell tumor. A diffuse sarcomatoid growth is focally associated with tubular formation.

网状型Sertoli-Leydig细胞瘤

- 当肿瘤含有类似于睾丸网的、吻合的裂隙样腔隙的明显区域时，称为“网状型Sertoli-Leydig 细胞瘤”。网状型小管是不见于高分化Sertoli-Leydig 细胞瘤。网状型肿瘤一般发生于较年轻患者，男性化较少见。网状结构的形态学变化较大，从被覆立方或柱状上皮的裂隙样腔隙，至乳头状结构区域，直至被覆扁平形细胞的多囊性结构伴筛孔状腔隙。

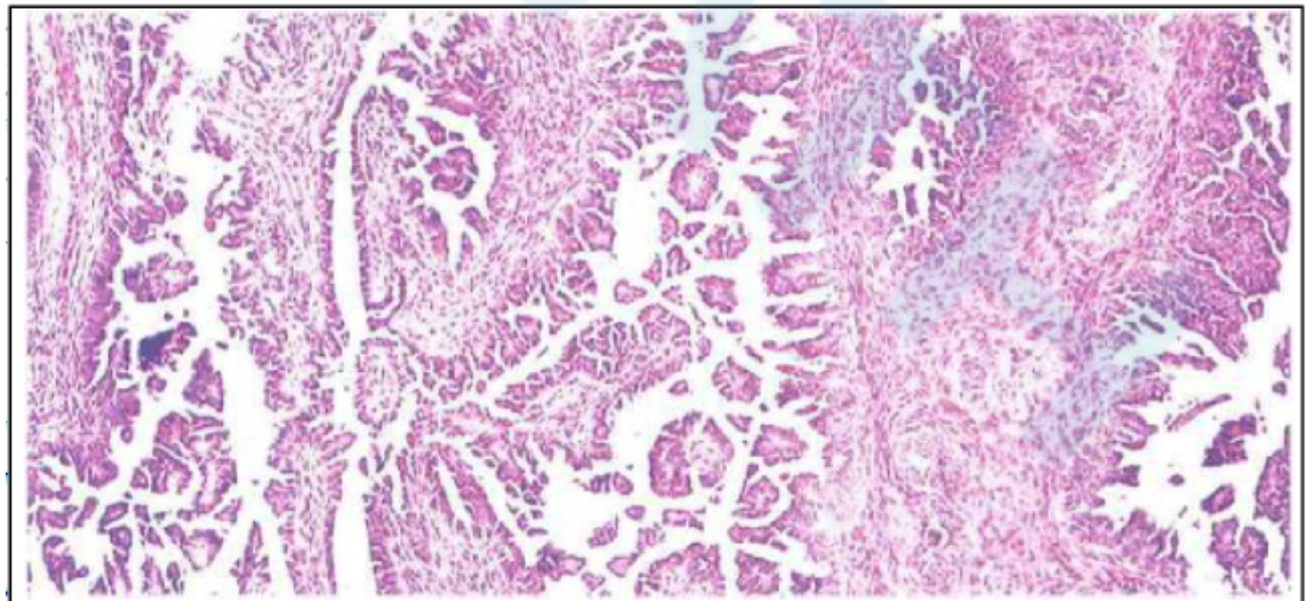


图 1.52 网状型 Sertoli-Leydig 细胞瘤。旺炽的网状结构类似浆液性乳头状肿瘤。

Sertoli-Leydig细胞瘤伴异源性成分

- 高达20%的Sertoli-Leydig 细胞瘤可见异源性成分。
- 包括上皮性和/或间叶性组织和这些成分形成的肿瘤。
- 仅见于中分化或低分化或网状型肿瘤。
- 异源性成分

--最常见是肠型黏液性上皮，其形态通常温和，但可能表现为增殖、交界性改变和癌。

异源性肝细胞 → 血清甲胎蛋白（AFP）升高。

--异源性间叶性成分更少见，通常由软骨或骨骼肌组成，通常细胞丰富，呈胎儿型。

免疫组化

- Vimentin、CK、α-inhibin 和calretinin 均为阳性，性索和间质区域具有不同的表达强度。
- Sertoli-Leydig细胞瘤表达CD56、SF-1 和WT-1、CD99
- 至少50%的病例表达FOXL2。Sertoli-Leydig细胞瘤中的Leydig 细胞不表达或微弱表达FOXL2、WT-1 和CD99，但是表达Melan-A。
- Dicer1 免疫组化显示Sertoli细胞强阳性，而Leydig 细胞弱阳性。
- Leydig细胞不表达CK 和AFP，但表达vimentin 和 α-inhibin。

遗传易感性

- DICER1 是编码一RNase III 核糖核酸内切酶的基因，其突变见于60%的 Sertoli-Leydig 细胞瘤。

预后和预测因素

- Sertoli-Leydig 细胞瘤的总体预后好，但与肿瘤分级呈显著相关。
- 高分化肿瘤的生存率接近100%。伴或不伴异源性成分的中分化肿瘤中，临床恶性者大约仅占病例的10%。出现网状成分可能对中分化肿瘤的预后有轻微不利的影响，但缺乏明确证据。低分化肿瘤和少数中分化肿瘤可能有恶性行为；常在2 年内复发和腹腔复发。
- 肿瘤破裂、出现间叶性异源性成分、分期≥ II 期具有不利的临床结局。

DICER1

- *DICER1*基因定位于14q32.13，编码一种具有1922个氨基酸残基的核糖核酸酶。Dicer1酶能够识别具有茎环结构的单链前体miRNA(precursor miRNA，pre-miRNA)或双链RNA（double strand RNA，dsRNA），并将其切割成21～23nt的成熟miRNA或siRNA。
- *DICER1*基因表达异常与肿瘤、多发性硬化症、突发性听力丧失等疾病密切相关。
- *DICER1*综合征是一种由于DICER1胚系突变导致的常染色体显性遗传性，有肿瘤形成倾向的疾病。
- 家族性多结节性甲状腺肿

  儿童期胸膜肺母细胞瘤

  宫颈胚胎性横纹肌肉瘤

  卵巢Sertoli-Leydig cell 肿瘤

  肾囊性肾瘤

FOXL2

- FOXL2是叉头转录因子超家族成员,FOXL2基因是第一个被认定在维持卵巢功能方面发挥重要作用的常染色体基因,在眼睑和卵巢颗粒细胞表达,参与脊椎动物雌性性腺早期发育与分化,对卵泡生长发育起重要调节作用.其基因突变可致卵巢早衰、女性不孕.
- 超过90%的成年型粒层细胞瘤在F0XL2 基因（402C 至G）有错义的体系点突变。

OBJECTIVE

- The frequency of DICER1 mutations in SLCT ranges widely from 15% to 97%
- Occasional SLCT harbor the same FOXL2 mutation (p.C134W)

- the goal of this study was to create a molecular classifier of SLCT by integrating the clinicopathologic features of the current WHO classification system with DICER1 and FOXL2 mutation status.

MATERIALS AND METHODS

- Patient Cohort：42 SLCTs.
- Immunohistochemistry： tissue microarray FOXL2 IHC
- DICER1 Mutation Analysis by Sanger Sequencing
- DICER1 Mutation Analysis by MiSeq
- FOXL2 Mutation Analysis
- Statistical Analysis

RESULTS

TABLE 1. Clinicopathologic and Molecular Features of SLCT[1]

Case	Age	Grade	Heterologous Elements	Retiform Pattern	*FOXL2* Mutation Status	*DICER1* Mutation Status	Elevated Androgens	Androgenic Symptoms
1	16	Poorly differentiated			WT	p.D1810V	Yes	Yes (AM)
2	80	Moderately differentiated			Mutant	WT	Unknown	No
3	74	Moderately differentiated			WT	WT	Unknown	Unknown
4	69	Well differentiated			WT	WT	Unknown	Yes (H)
5	57	Moderately differentiated		Yes	WT	p.D1709N	No	No
6	23	Moderately differentiated	Yes		WT	p.E1813K	Unknown	No
7	69	Moderately differentiated			WT	WT	Unknown	No
8	23	Well differentiated			WT	Failed x2	Yes	Yes (H)
9	44	Moderately differentiated			WT	p.E1813K	Yes	Yes (DV, H, AC)
10	82	Moderately differentiated			Mutant	WT	Unknown	No
11	27	Moderately differentiated	Yes		WT	p.E1813G	Unknown	No
12	18	Moderately differentiated			WT	p.D1810H	Yes	Yes (C, H, AM)
13	16	Moderately differentiated	Yes		WT	p.Y1701X; p.E1705K	No	No
14	17	Moderately differentiated			WT	WT	Yes	Yes (AM)
15	47	Moderately differentiated		Yes	WT	p.D1709E	Unknown	No
16	34	Moderately differentiated			WT	WT	Yes	Yes (C, AM)
17	62	Poorly differentiated			Mutant	WT	Unknown	No
18	16	Moderately differentiated			WT	p.D1709N	Unknown	No
19	78	Moderately differentiated			Mutant	WT	Unknown	No
20	24	Moderately differentiated			WT	WT	Unknown	Unknown
21	35	Moderately differentiated	Yes		WT	p.E1813D; p.D1709N	Yes	Yes (AM)
22	62	Moderately differentiated			WT	p.D1709N	No	No
23	50	Moderately differentiated			WT	WT	Unknown	No
24	90	Moderately differentiated			Mutant	WT	Unknown	Unknown
25	17	Moderately differentiated	Yes		WT	p.E1813K	Yes	Yes (DV)
26	88	Moderately differentiated			Mutant	WT	Unknown	No
27	66	Moderately differentiated			WT	WT	Unknown	No
28	21	Moderately differentiated			WT	p.E1705K	Unknown	Yes (AM)
29	52	Well differentiated			WT	WT	Unknown	No
30	73	Poorly differentiated			WT	WT	Unknown	No
31	32	Moderately differentiated			WT	WT	No	Yes (C, DV, H, AM)
32	60	Poorly differentiated			WT	WT	Unknown	Unknown
33	44	Moderately differentiated	Yes		WT	p.E1813G	Unknown	Unknown
34	79	Poorly differentiated			Mutant	WT	Unknown	Unknown
35	54	Poorly differentiated			Mutant	WT	Unknown	Unknown
36	16	Moderately differentiated			WT	p.D1709E	Unknown	Unknown
37	15	Moderately differentiated	Yes		WT	p.D1709V	Unknown	Unknown
38	35	Well differentiated			WT	WT	Unknown	Unknown
39	26	Moderately differentiated	Yes		WT	p.D1709N	Unknown	Unknown
40	51	Moderately differentiated			WT	WT	Unknown	Unknown
41	44	Well differentiated			WT	WT	Unknown	Unknown
42	61	Moderately differentiated			WT	p.D1709N	Yes	Yes (H)

AC indicates acne; AM, amenorrhea; C, clitoromegaly; DV, deepening voice; H, hirsutism; WT, wild type.

Cohort Description and Histopathologic Features

- Median age for the cohort was 47 years (range, 15 to 90 y).
- 5 tumors (11%) were well differentiated, 31 (74%)moderately differentiated, and 6 (14%) poorly differentiated
- There was no statistically significant difference in the age distribution of patients with each tumor grade(well differentiated: median, 44y; range, 23 to 69y; moderately differentiated: median, 44 y; range, 15 to 90 y; poorly differentiated: median, 61 y; range, 16 to 79 y).
- 8 tumors (19%) contained heterologous elements (7 epithelial/gastrointestinal mucinous and 1 mesenchymal), and 2 (5%)displayed retiform differentiation
- Heterologous elements and retiform features were mutually exclusive; all 10 tumors with these features were moderately differentiated. Nine of 10 patients with retiform features or heterologous elements occurred in premenopausal patients (median, 26.5 y; range, 15 to 57 y).

DICER1 Mutation Status

DICER1 mutations were identified in 18/41(44%) successfully genotyped tumors (17 moderately differentiated, 1 poorly differentiated; 49% of moderately and poorly differentiated tumors), including all 10 tumors with heterologous elements or retiform pattern.

FOXL2 Mutation Status

- FOXL2 mutation was identified in 8/42 (19%) tumors (8 moderately and 3 poorly differentiated).
- DICER1 and FOXL2 mutations were mutually exclusive in 41 cases successfully tested for both mutations.

DICER1/ FOXL2 wild type

- 15/41(37%)
- All 5 well-differentiated , 8moderately and 2poorly differentiated.

Immunohistochemistry

- All tumors expressed the sex cord-stromal tumor marker FOXL2 by IHC
- The sertoliform component was positive, and the Leydig cell component was weakly positive or negative.

TABLE 2. Statistical Analysis of Clinical, Pathologic and Molecular Features in SLCT[1]

Clinicopathologic Features	Statistical Comparison	Results	*P*
Younger age (mean [median]) (y)	*DICER1* vs. *FOXL2*	31.2 (24.5) vs. 76.6 (79.5)	<0.0001
	DICER1 vs. WT/WT	31.2 (24.5) vs. 50 (51)	0.005
	WT/WT vs. *FOXL2*	50 (51) vs. 76.6 (79.5)	0.0006
	DICER1, WT/WT, *FOXL2*	31.2 (24.5) vs. 50 (51) vs. 76.6 (79.5)	<0.0001
	DICER1 vs. WT/WT(MD-PD)	31.2 (24.5) vs. 50 (51)	0.02
	WT/WT(MD-PD) vs. *FOXL2*	50 (51) vs. 76.6 (79.5)	0.003
	DICER1, WT/WT(MD-PD), *FOXL2*	31.2 (24.5) vs. 50 (51) vs. 76.6 (79.5)	<0.0001
	Well vs. moderately differentiated	44.6 (44) vs. 44.5 (44)	0.99
	Well vs. poorly differentiated	44.6 (44) vs. 57.3 (61)	0.31
	Moderately vs. poorly differentiated	44.5 (44) vs. 57.3 (61)	0.24
	Well vs. moderately vs. poorly differentiated	44.6 (44) vs. 44.5 (44) vs. 57.3 (61)	0.48
Abnormal bleeding	*FOXL2* vs. WT	2/5 (40%) vs. 2/24 (8%)	0.13
Androgenic symptoms	*DICER1* mut vs. WT	7/14 (50%) vs. 4/14 (29%)	0.22
	DICER1 mut vs. WT(MD-PD)	7/14 (50%) vs. 3/12 (25%)	0.18
	DICER1 vs. *FOXL2*	7/14 (50%) vs. 0/7 (0%)	0.03
Elevated serum androgen levels	*DICER1* vs. WT	6/9 (66.7%) vs. 2/3 (66.7%)	0.76
Heterologous elements or retiform pattern	Pre vs. post menopausal	9/23 (39%) vs. 1/19 (5%)	0.01
	DICER1 vs. WT	10/18 (55.6%) vs. 0/23 (0%)	<0.0001
Leydig cells conspicuous	*DICER1* vs. *FOXL2*	7/18 (38.9%) vs. 0/8 (0%)	0.05
	WT/WT vs. *DICER1*	11/15 (73%) vs. 7/18 (39%)	0.05
	WT/WT vs. *FOXL2*	11/15 (73%) vs. 0/10 (0%)	0.0003
	WT/WT (MD-PD) vs. *DICER1*	8/11 (73%) vs. 7/18 (39%)	0.08
	WT/WT (MD-PD) vs. *FOXL2*	8/11 (73%) vs. 0/10 (0%)	0.0008

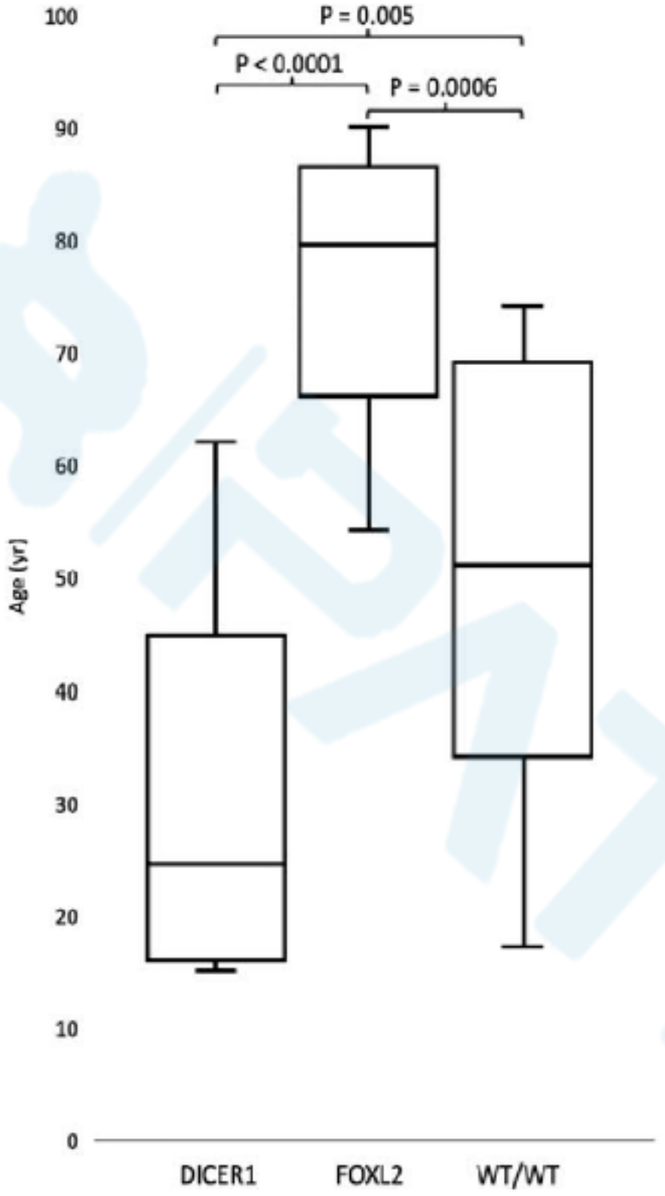
DICER1<WT/WT<*FOXL2*

DICER1

DICER1

FOXL2

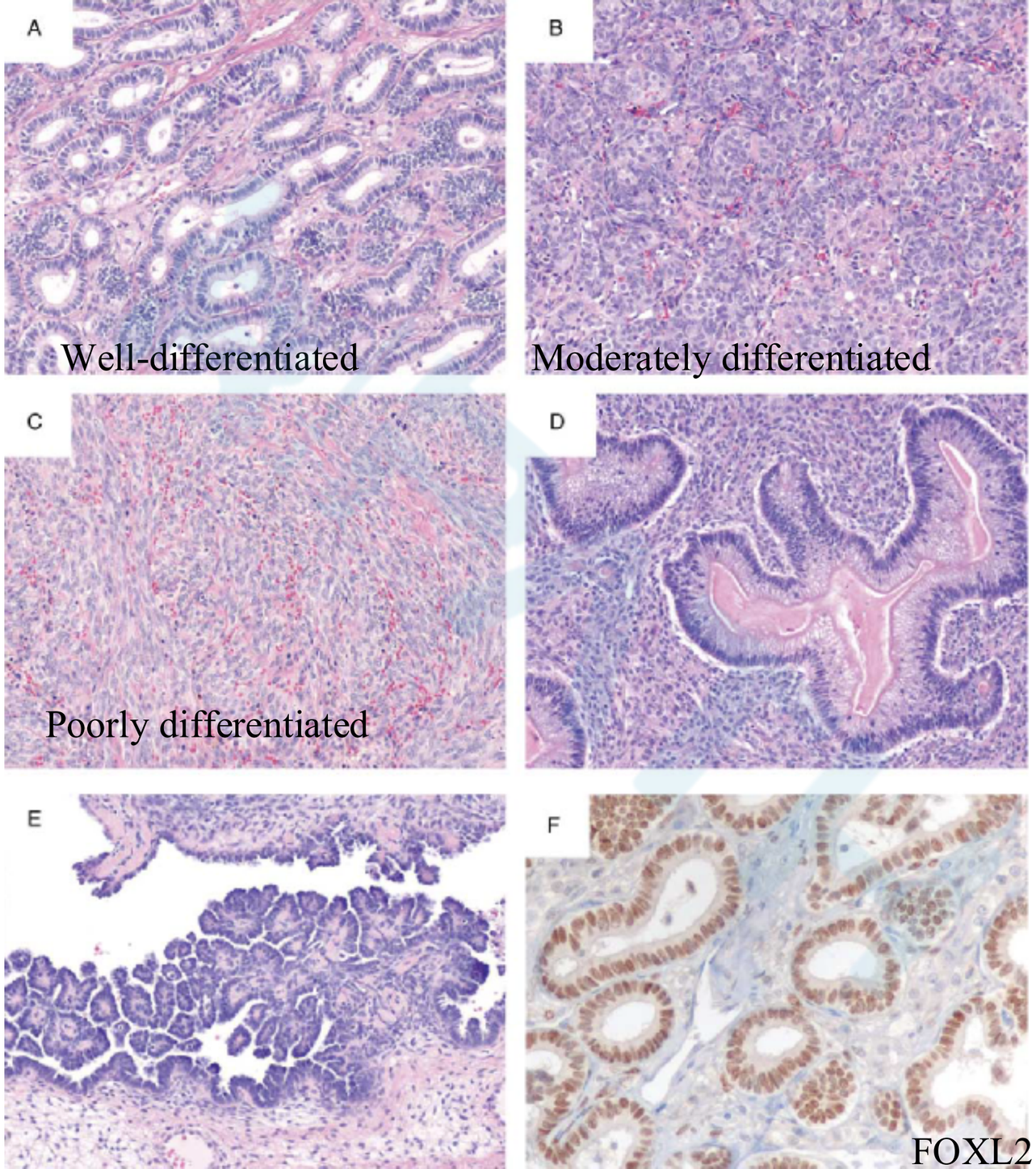
DICER1 indicates tumors harboring somatic mutation in the RNase IIIb domain of *DICER1*; *FOXL2*, tumors harboring *FOXL2* c.402C>G mutation; MD-PD, only moderately differentiated and poorly differentiated tumours used in the comparison; WT/WT, tumors wild type for both *DICER1* and *FOXL2*; WT, tumors wild type for the gene in the comparison.

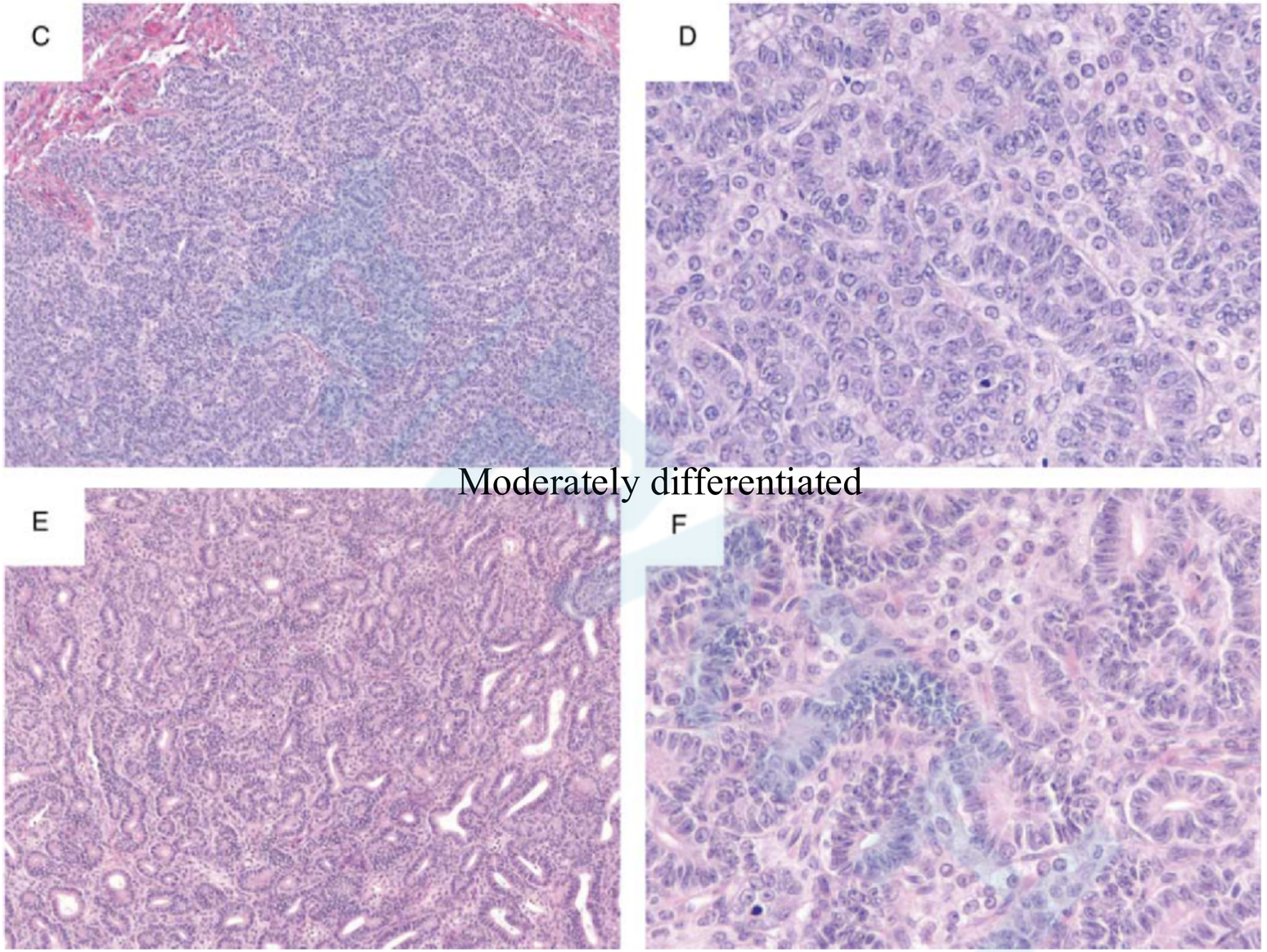


IGURE 4. Mutation status according to patient age at pre-entation. Patients' age at presentation were plotted for each nolecular subtype: *DICER1*-mutated, *FOXL2*-mutated, or wild ype for both genes (WT/WT). Mean ages were 24.5 years range, 54 to 90 y, DICER1), 79.5 years (range, 51 to 90 y, FOXL2), and 51 years (range, 17 to 74 y, WT/WT).

Outcome

- Outcome data were available for 31 patients.
- Four patients (13%) died, including 1 from recurrent disease(case 5, DICER1 mutant, 21 mo survival). The other 3 patients died of unrelated causes (cases 2 and 26, FOXL2 mutant, and case 30, DICER1/FOXL2 wild type).
- Three patients experienced recurrences (case 5, DICER1 mutant, at 10 mo; case 20, DICER1/FOXL2 wild type, at 58 mo; and case 36, DICER1 mutant, at 134 mo).


A
Well-differentiated
B
Moderately differentiated
C
Poorly differentiated
D
E
F
FOXL2



C–F, Tumor with FOXL2 c.402C>G mutation (case 11). All tumors lacking DICER1 mutations showed varying degrees of sertoliform differentiation or primitive ovarian stroma interspersed with Leydig-like cells. None displayed definitive features of adult granulosa cell tumor or other sex cord-stromal tumors.

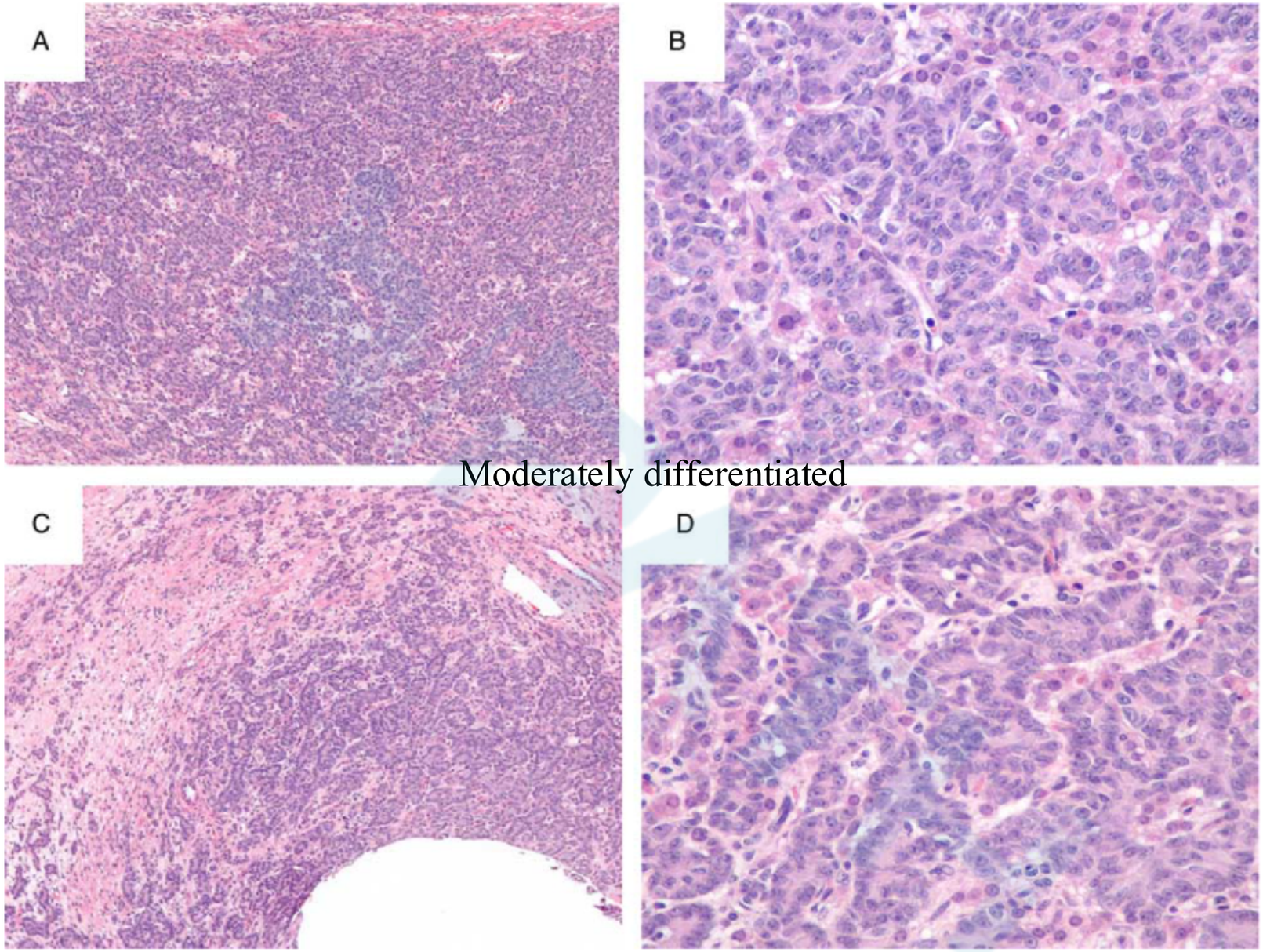
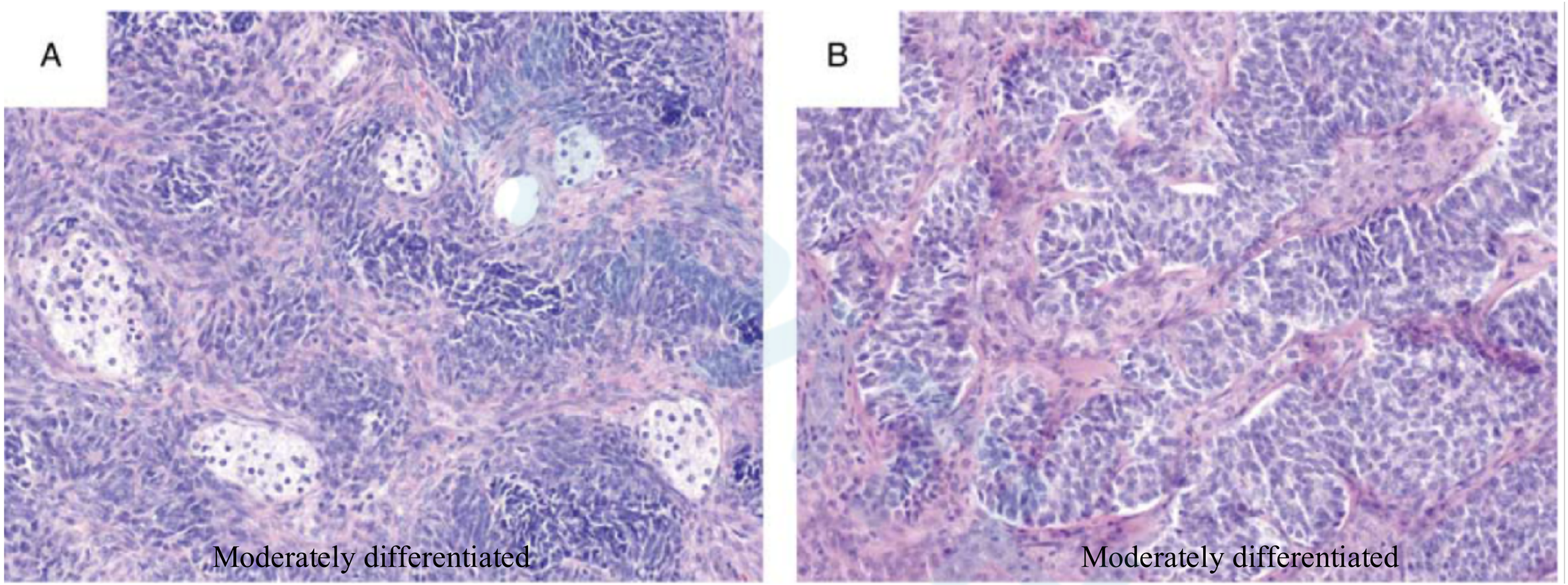


FIGURE 3. Histopathology of a FOXL2-mutant tumor (case 7). The slides showed a predominance of immature and closed tubules with conspicuous interspersed luteinized stromal/Leydig cells containing round to ovoid nuclei with conspicuous small nucleoli, and bright eosinophilic cytoplasm.



A and B, Examples of tumors wild type for DICER1 and FOXL2 (cases 3 and 31, respectively).

DISCUSSION

- This is the first study that identifies 3 molecular subtypes of ovarian SLCT

--DICER1 mutant subtype

--DICER1/ FOXL2 wild type

--FOXL2-mutant subtype

DICER1 mutant subtype

- Somatic mutations in the RNase IIIb domain of DICER1 were found in 44% of samples overall
- moderately or poorly differentiated,
- displaying heterologous elements or retiform pattern
- generally younger
- more often presented with androgenic symptoms.

FOXL2-mutant subtype

- FOXL2 mutations were identified in 19% of cases, all of which were moderately differentiated.
- postmenopausal
- more likely to present with abnormal bleeding
- and had less conspicuous Leydig cells.

DICER1/ FOXL2 wild type

- All 5 well-differentiated tumors were wild type for FOXL2 and 4 of 5 were wild type for DICER1
- intermediate age,
- no retiform or heterologous elements,

- It is unclear at this time if the SLCT molecular subtypes are prognostic, as our analysis lacked statistical power to determine whether clinical outcome correlated with mutation status.

SUMMARY

- 3 molecular subtypes of SLCT with characteristic clinicopathologic features
- The only definitive predictors of mutation status in our cohort were the presence of heterologous elements or retiform pattern, which predicted DICER1 mutation, and low tumor grade, which predicted a lack of either DICER1 or FOXL2 mutation
- Other features such as age and presenting symptoms, while statistically significant in their correlation with mutation status, did not offer sufficient predictive power to be useful predictors for an individual patient in a clinical setting.