

Fluorescent In Situ Hybridization Analysis for 12p Alterations in Sarcomatoid Yolk Sac Tumors

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汇报人：刘家艳

背景

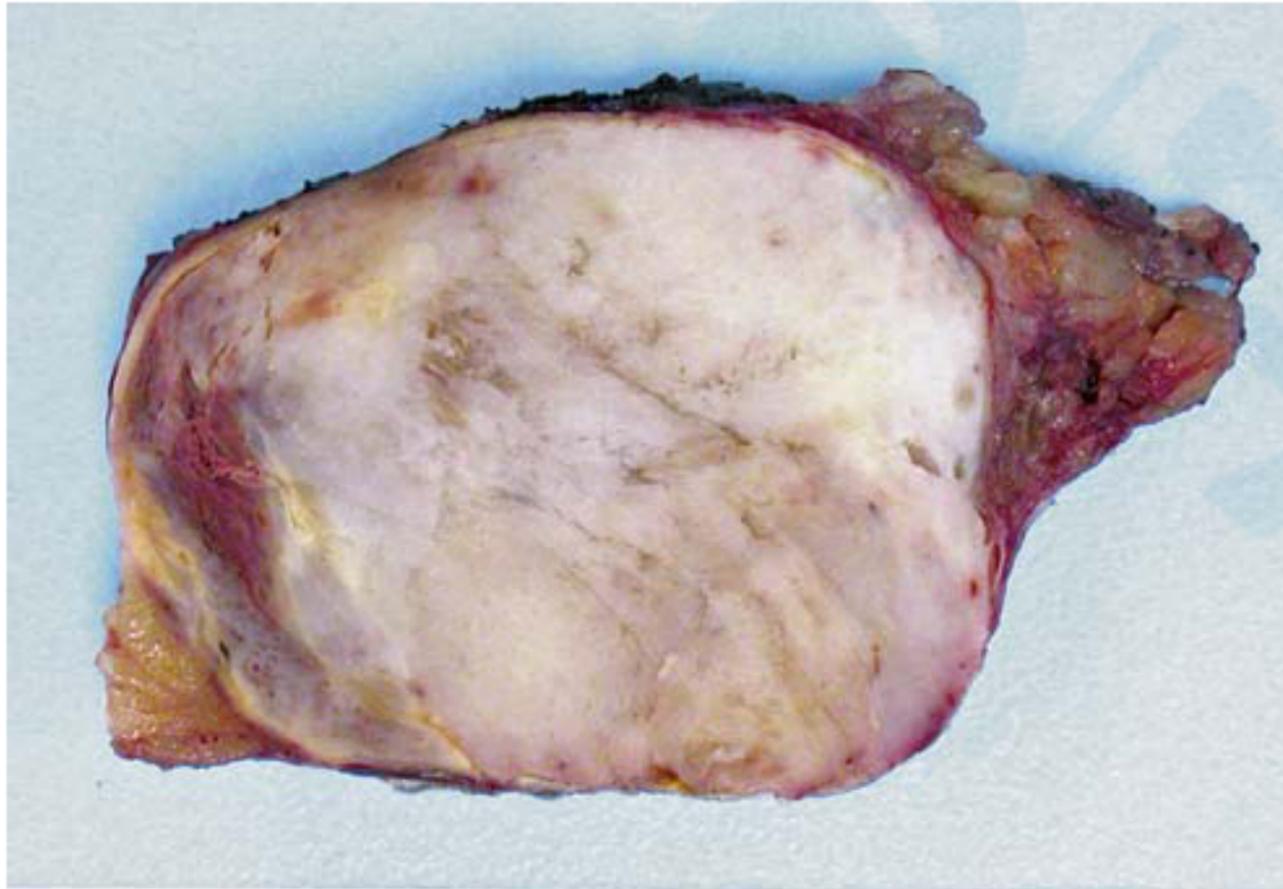
- 起源于原位生殖细胞肿瘤的恶性GCTS的主要遗传学改变是染色体12p异常，包括等臂染色体12p[i(12)p]和其他形式的染色体改变。一些研究证实，在转移性GCTS中，以及由此发生的大多数体细胞型恶性肿瘤中，都可以检测到12p的过度表达。
- 睾丸生殖细胞肿瘤（TGCTS）伴肉瘤样变发生于<4%的病例中，最常见的肉瘤类型是横纹肌肉瘤，也可以是血管肉瘤、平滑肌肉瘤、软骨肉瘤等。常见于肿瘤晚期复发病例中，往往治疗困难，常表现为化疗耐药，多发复发/转移，预后差。

Many Postchemotherapy Sarcomatous Tumors in Patients
With Testicular Germ Cell Tumors Are Sarcomatoid
Yolk Sac Tumors
A Study of 33 Cases

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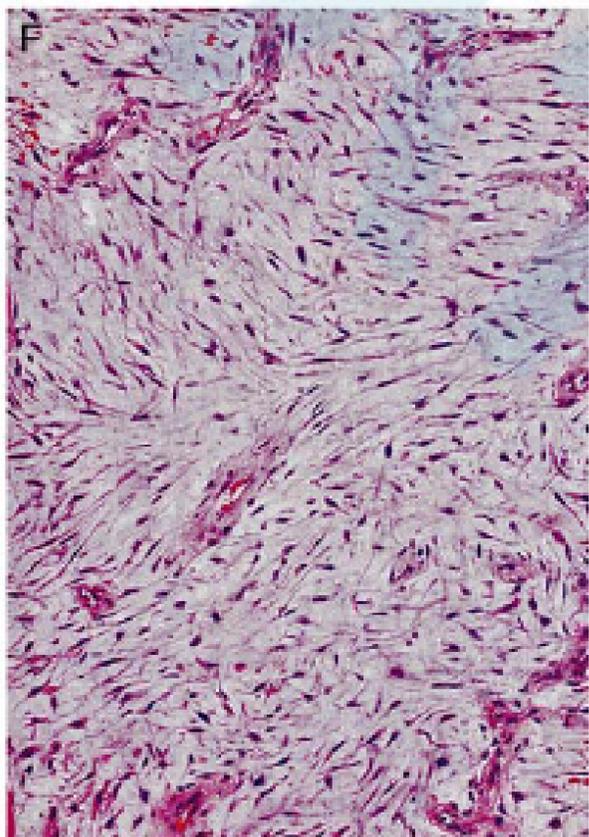
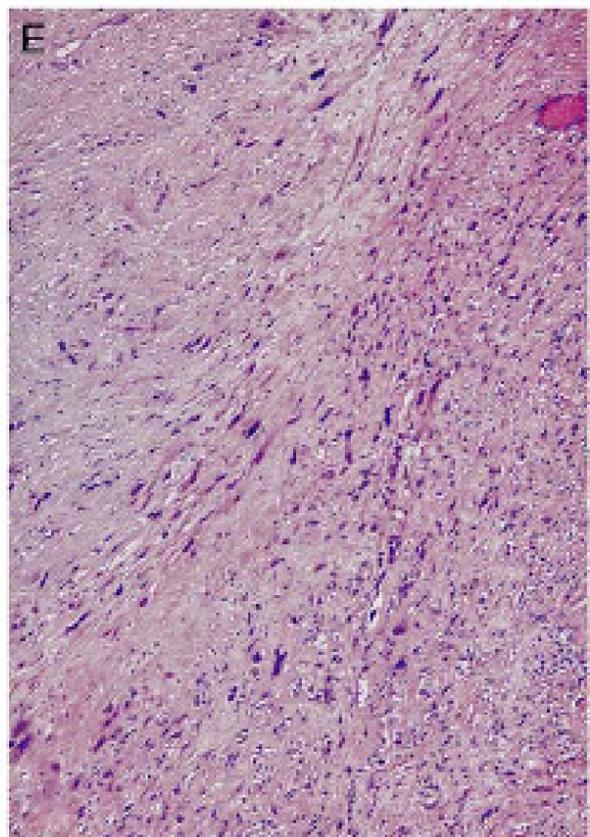
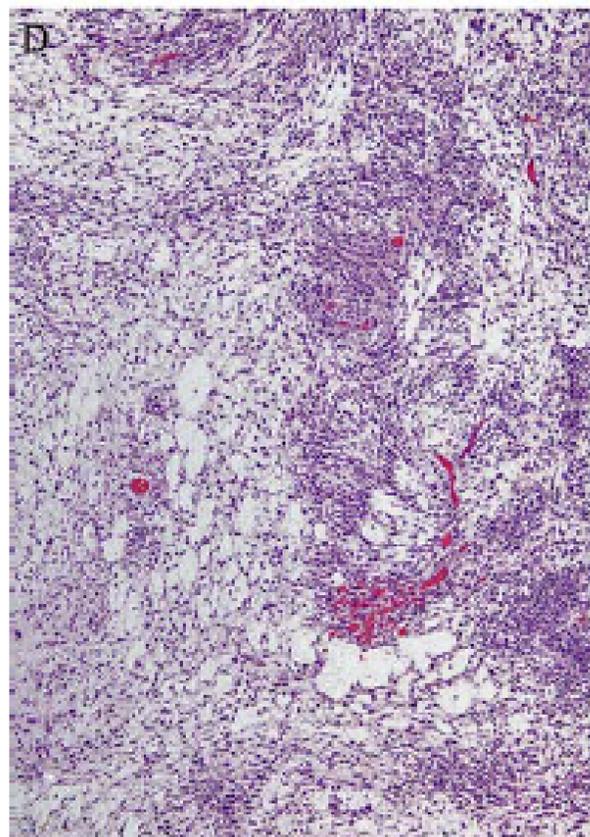
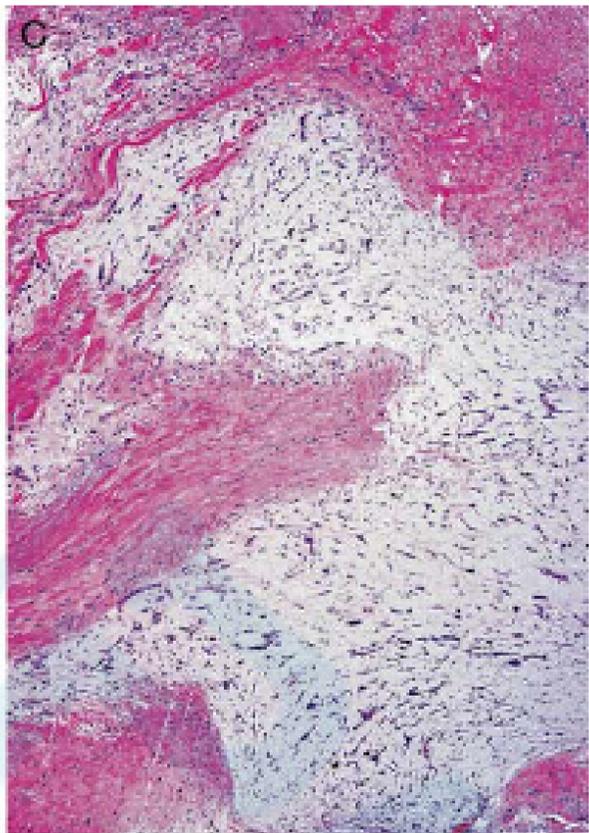
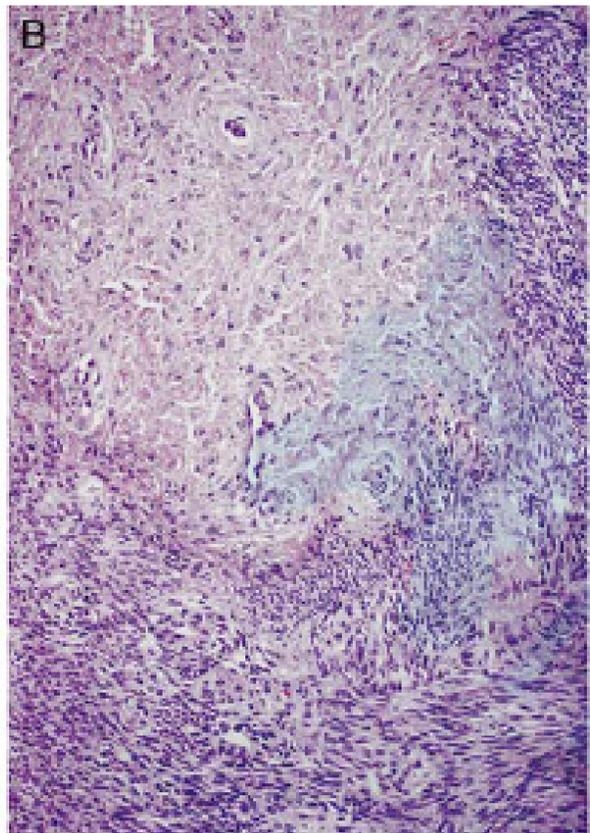
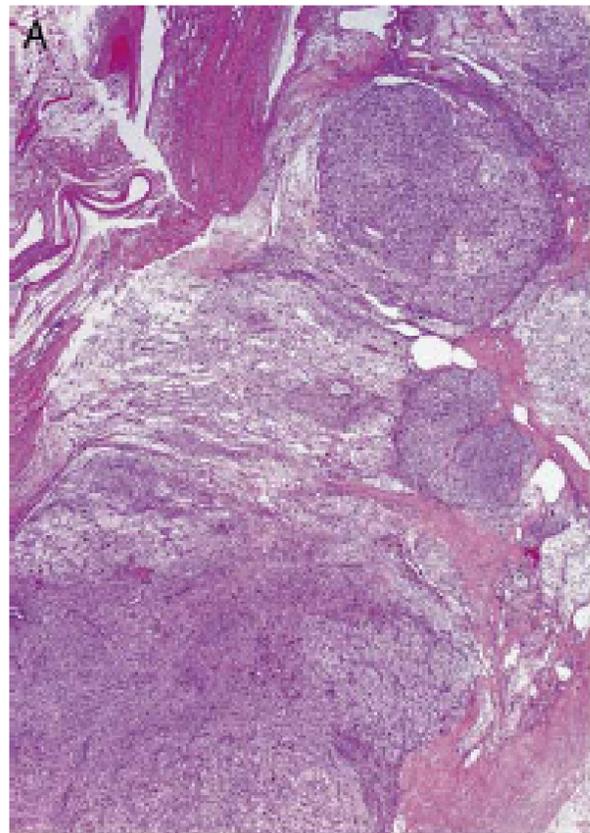
- 研究表明，化疗后的TGCT患者发生肉瘤转化有很高比例是肉瘤样卵黄囊瘤（SYSTs），通常发生在诊断TGCT数年后，血清AFP正常或轻微升高，高级别时往往表现为较高的侵袭性。文中对其形态特征和免疫表型进行详细描述。
- SYST中12p是否过度表达？

肉瘤样卵黄囊瘤 (SYST)

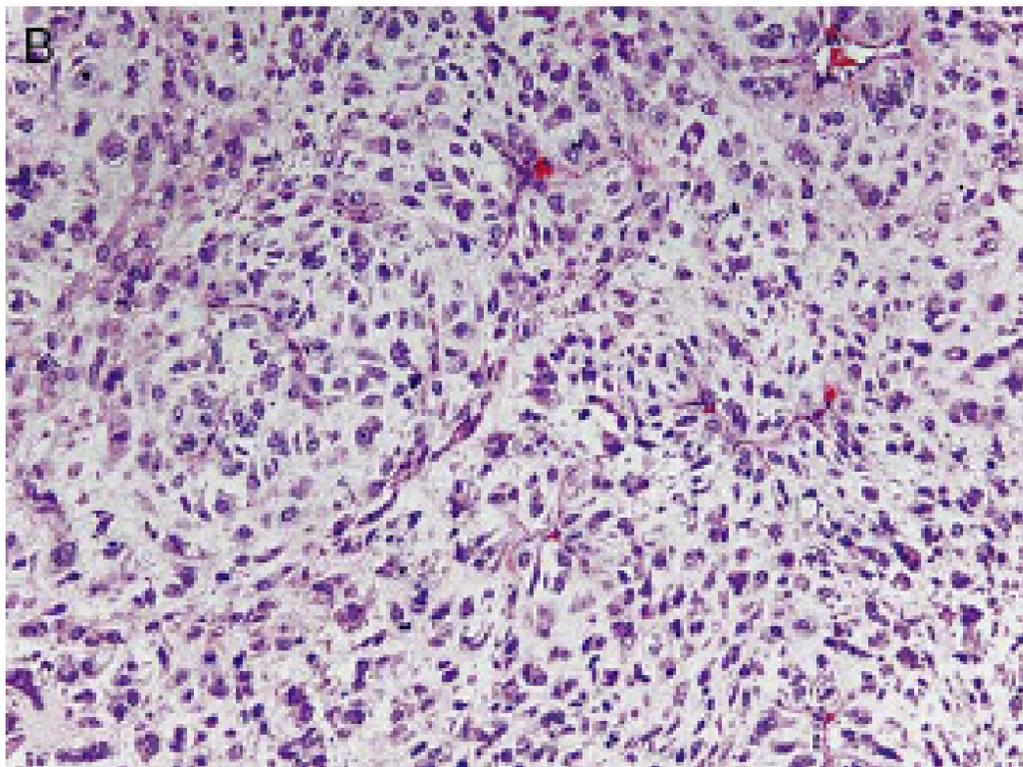
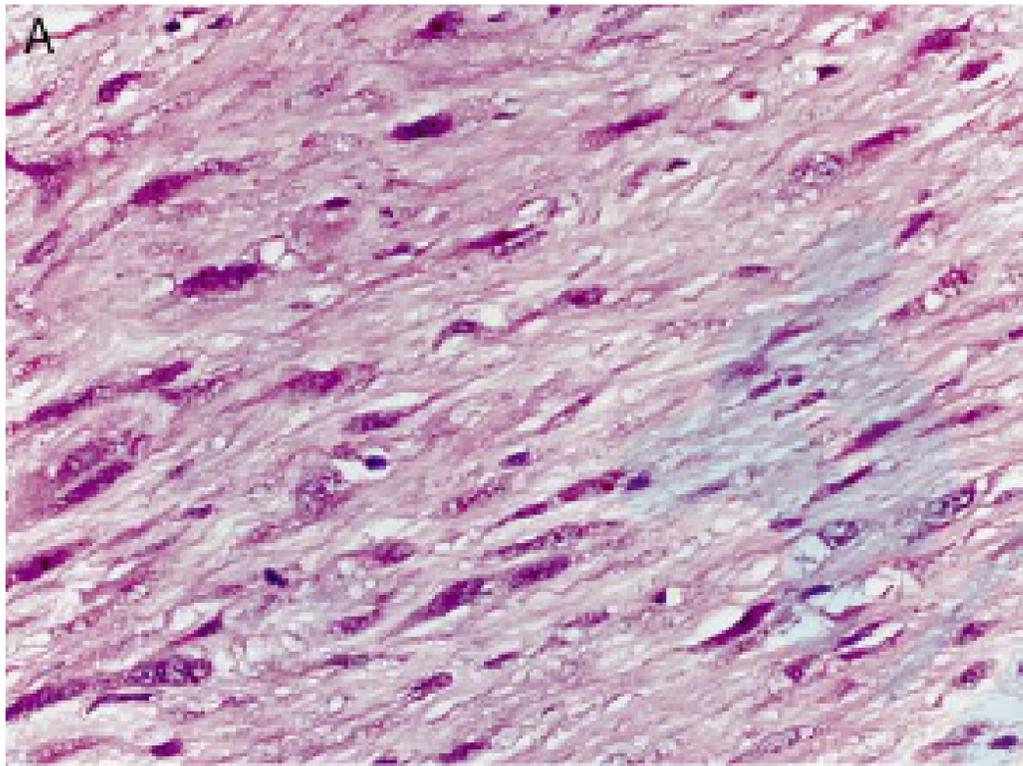


- 大体表现为边界清楚的肿块，结节状或分叶状，有时外周可有明显的纤维性包裹
- 瘤体呈实性，致韧，褐色或灰白色
- 可有局灶囊性变和出血
- 少数以黏液样或胶冻状为主

FIGURE 1. SYST on gross examination are well circumscribed, lobulated, and typically have firm, tan to white cut surfaces, frequently with foci of myxoid change.



- 低倍镜下，大多数SYSTs呈结节状或分叶状生长
- 疏松区与致密区交替
- 黏液样区域与富含胶原间质区域有界限鲜明，类似于低度恶性纤维粘液样肉瘤
- 间质为黏液样、纤维性和/或纤维黏液样
- 缺乏明显的束状生长模式



- 瘤细胞呈梭形或上皮样形态
- 以梭形细胞为主，可表现为纯梭形细胞，也可表现为梭形细胞和上皮样细胞混合

FIGURE 3. Nuclear morphology and cell shape in SYST. A, Spindled cells with elongated nuclei are common in SYST. B, Epithelioid cells with plump, rounded nuclei were less common, although in 3 cases this pattern predominated.

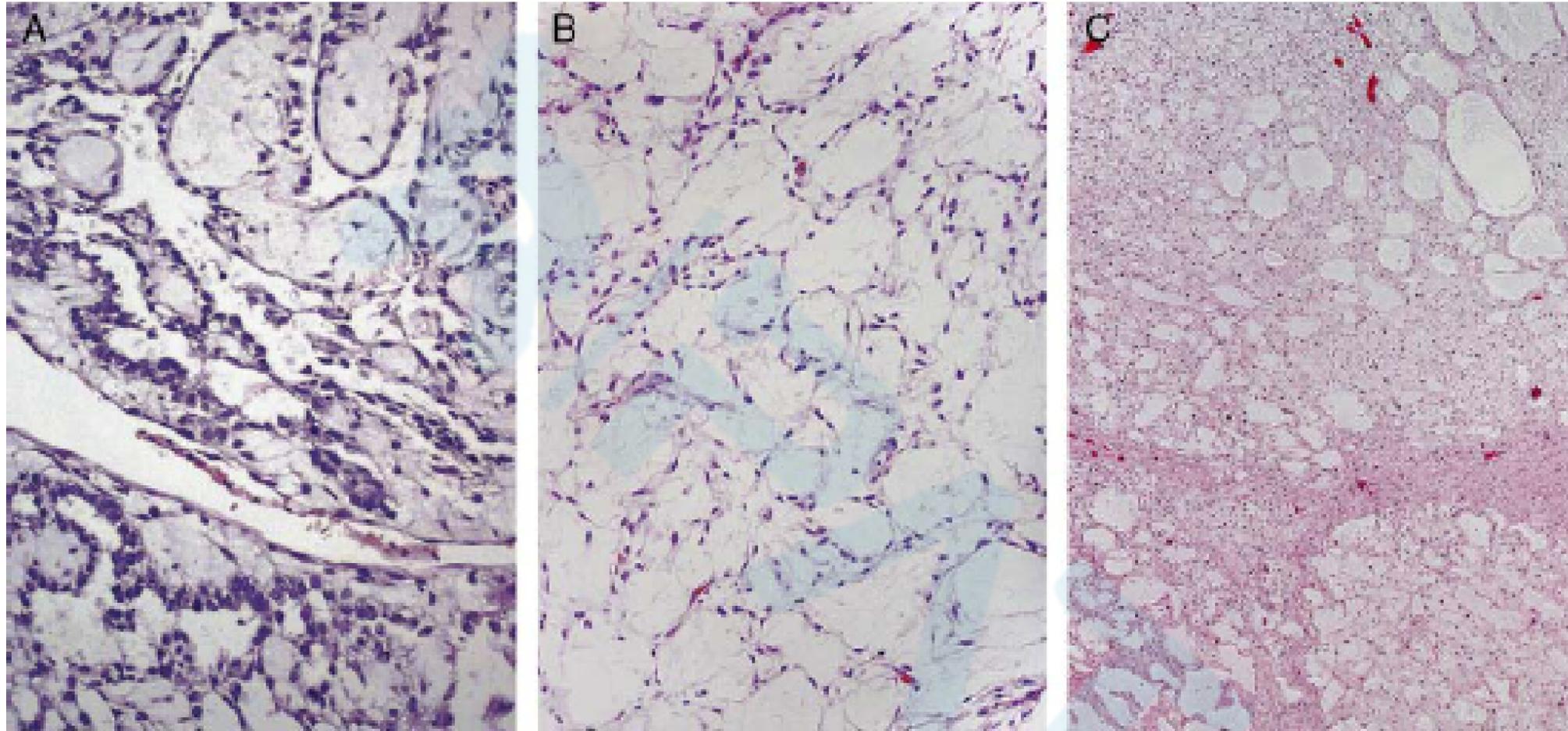


FIGURE 4. Myxoid tumor ringlets and microcystic stromal change in YST and SYST. A, Classic YST may occasionally form myxoid tumor ringlets. SYST commonly formed similar tumor ringlets (B) and in many cases were associated with more severe microcystic change (C).

有些可呈微囊性外观，无细胞囊腔被肿瘤细胞包围，形成独特的肿瘤细胞环，也可见于经典的YST

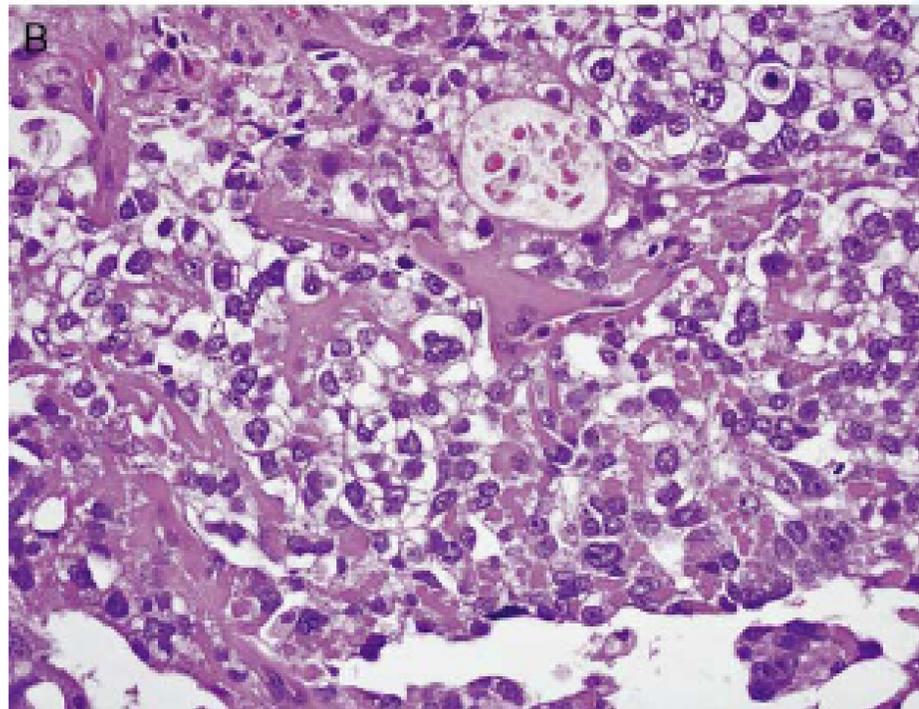
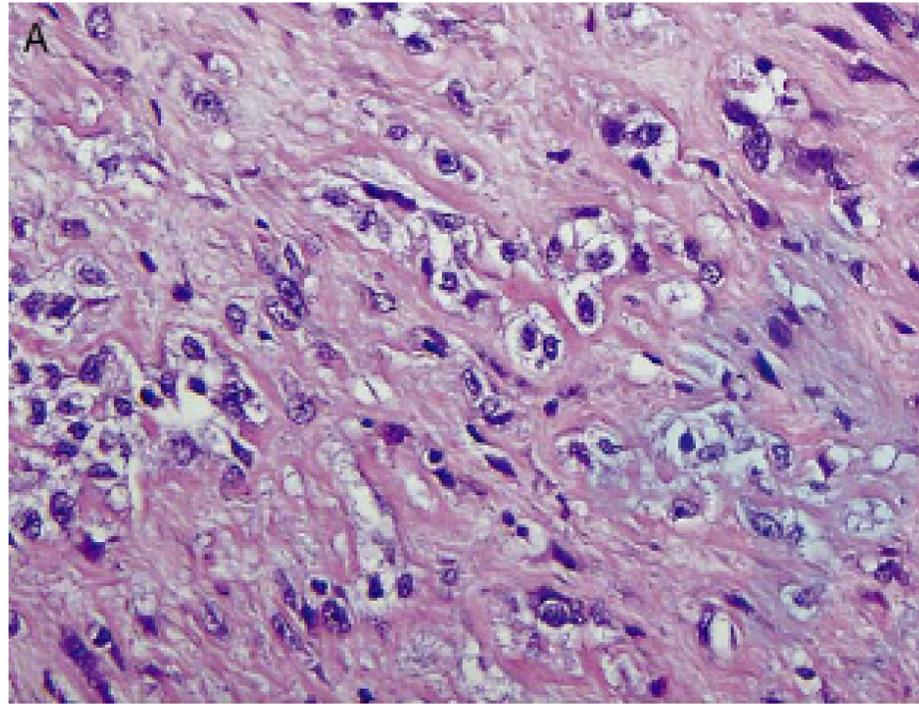


FIGURE 5. Parietal differentiation in SYST. A, In over half the cases, there was at least focal intercellular dense hyaline basement membrane-like material (“parietal” differentiation), most often associated with areas of epithelioid morphology and mimicking chondroid or osseous differentiation. B, Classic YST can also have varying amounts of parietal differentiation.

- 超过半数病例中，至少有局部可见细胞间致密的嗜酸性基底膜样物质，类似“体壁”分化 (“parietal” differentiation)，通常与上皮样形态和类似软骨样或骨分化的区域有关
- 经典的YST也可以有不同数量的“体壁”分化

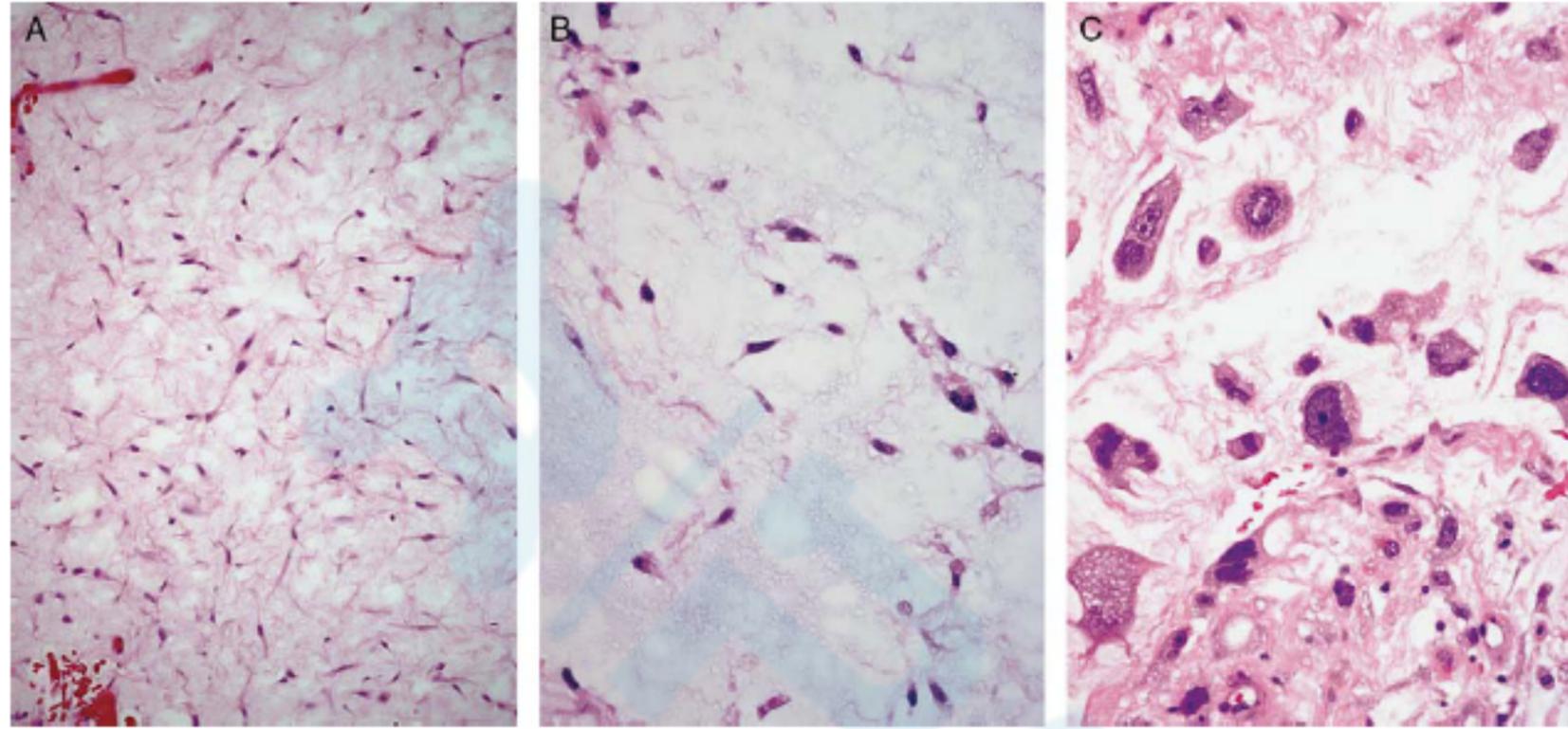


FIGURE 6. Cytologic atypia in SYST. Atypia ranged from mild (A), moderate (B), to severe (C), with most cases showing at least focal severe atypia.

- 核非典型性从轻度、中度到重度不等
- 重度不典型表现为核增大、不规则、泡状染色质、核仁明显
- 可见多形性瘤巨细胞、局灶性坏死
- 核分裂指数不等， $<1-39/10\text{HPF}$

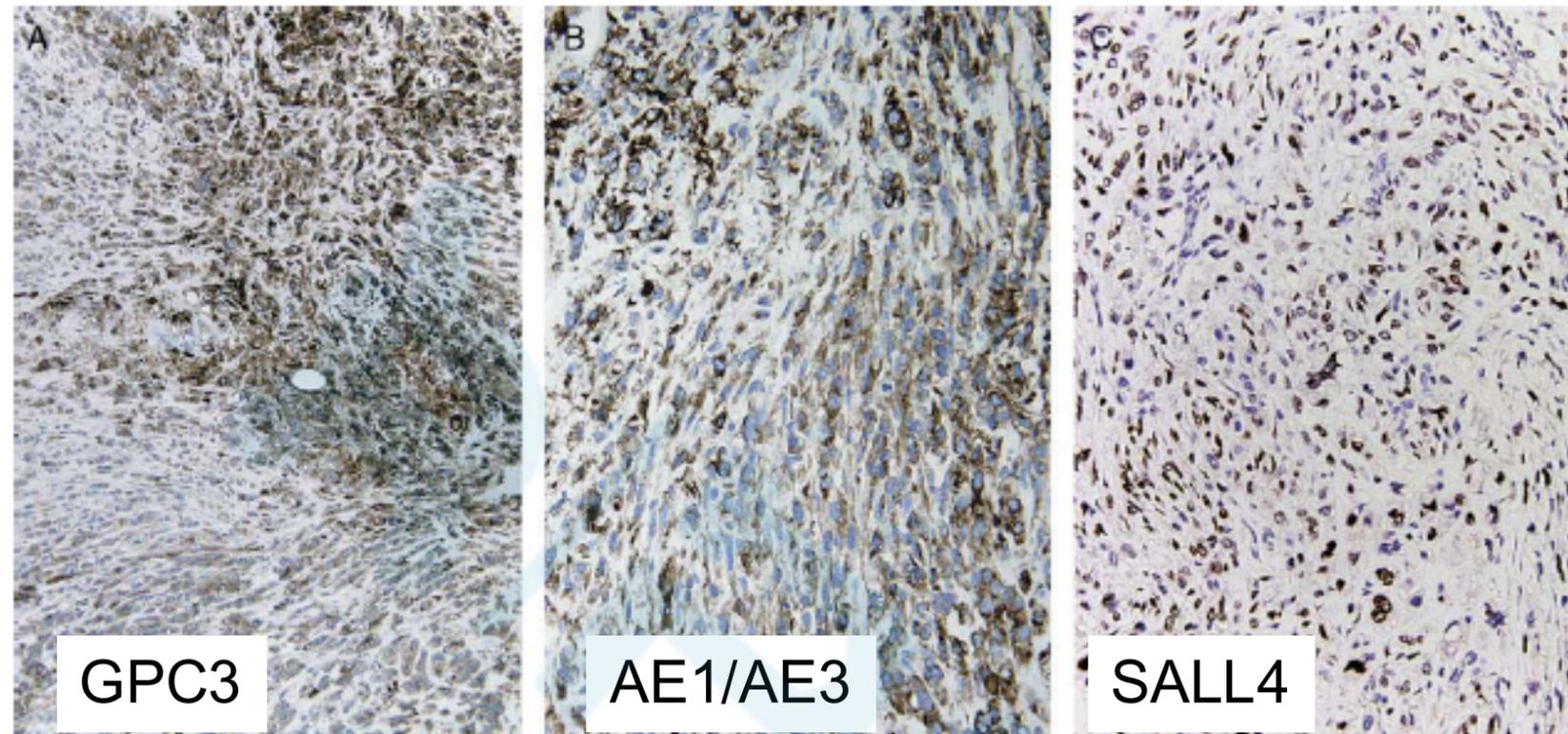


FIGURE 7. Immunohistochemical features of SYST. By definition, all SYSTs were positive for GPC3 (A) and AE1/AE3 (B). The majority of cases also demonstrated nuclear positivity for SALL4 (C).

- 所有SYST均表达AE1/AE3和GPC3
- 部分病例可表达SALL4、CD34
- 约一半病例表现出平滑肌分化 (desmin , caldesmon ,SMA阳性)
- 不表达AFP, MUC4, GFAP, myogenin

材料与amp;方法

- 筛选1994至2018年间，发生SYST的TGCTS病例
- 大多数病例(15/16)来自于先前有TGCTS病史的肉瘤样肿瘤的形态学和免疫组化特征的研究中。本研究中的所有肿瘤先前已根据组织学和免疫组化特征证实为SYST
- 由2名病理学家对HE染色切片进行形态学分析，包括肿瘤的分级，以及组织块的选择
- 采用FNCLCC分级系统：grade 1—低级别；grades 2 to 3—高级别

- 对来自11名患者的16例标本进行研究。其中9例标本分别来自9名患者(1例/人)；其余7例标本来自2名多次复发的患者，4例来自1名患者不同的复发或转移灶(#4)，3例来自另一患者(#1)。
- 免疫组化染色：AE1/AE3, Glypican 3 (GPC3), SALL4, CD34, P63, AFP, MUC4, GFAP, myogenin, desmin, caldesmon, SMA
- 对16例标本进行间期核-FISH检测，采用12号着丝粒对照探针（绿色信号）(D12Z3;Abbott Molecular-Vysis, Des Plaines, IL)和12p12.1探针（红色信号），对指定区域内60个细胞的信号模式进行评估。
- 12p过表达以12p的信号数与12号着丝粒的信号数之间的比率表示，比率 ≥ 1.3 认定为过表达。

TABLE 1. Clinopathologic Information of Patients With Sarcomatoid Yolk Sac Tumor

Case #	Age at Initial TGCT Diagnosis (y)	Subtype of TGCT	Prechemo AFP Level (ng/mL)	Pre-SYST AFP Level (ng/mL)	Time From Initial TGCT dx to SYST dx (mo)	Source of SYST	Interval From TGCT dx to Final Status (mo)	Interval From SYST dx to Final Status (mo)	SYST Grade	Clinical Status at Last Follow-up
1	36	EC+YST	Elevated (520)	Slightly elevated	132	Iliac artery and retroperitoneum	144	12	H	DOD
2	21	RPLN Bx = EC + YST; testis not reviewed	Elevated (specific)	Slightly elevated	132	Iliac artery and retroperitoneum	144	12	H	DOD
3	26	ND	Elevated	Normal	71	RPLND	90	19	H	DOD
4	28	EC+YST	Elevated	Normal	75	appendix	75	74	H	DOD
5	33	ND	Elevated	Normal	37	Intra-abd RPLND	44	7	H	DOD
6	23	ND	Elevated (2840)	Normal	113	Intra-abd RPLND	114	1	H	ANED
7	28	EC+YST	Elevated	Normal	9	(re-do) RPLND	69	60	L	ANED
8	33	EC+S	Elevated	Normal	9	(re-do) RPLND	69	60	H	DOD
9	24	EC+YST	Elevated (520)	Normal	9	(re-do) RPLND	69	60	H	ANED
10	20	T	Elevated	Normal	9	(re-do) RPLND	69	60	H	ANED
11	33	EC+S	Elevated	Normal	9	(re-do) RPLND	69	60	H	ANED

初次诊断 5人原发 化疗前8 SYST之 初次诊断 腹膜后10例；肺或纵隔3例；腹腔内3例 均46.5个月。#1和#4多次复发

1人原始诊断来自腹膜后淋巴结活检，YST是混合性GCT的一个组成部分

6/11人在诊断SYST后7--112个月死于疾病，平均39个月；4人无进展生存，随访时间1-74个月，平均36个月；1人在诊断SYST后39个月死于非相关疾病

SYST分级仅1例为低级别，截止文章发表时仍无进展生存，其余均为高级别

1人为单纯的畸胎瘤；1人为混合GCT（包括胚胎性癌和精原细胞瘤）

ANED indicates alive with no evidence of disease; Bx, biopsy; CC, choriocarcinoma; DOD, died of disease; DOU, died of unrelated cause; dx, diagnosis; EC, embryonal carcinoma; H, high; L, low; LN, lymph node; ND, no data; RPLN, retroperitoneal lymph node; RPLND, retroperitoneal lymph node dissection; S, seminoma; SC, supraclavicular; T, teratoma. *The likely precursor of the sarcomatoid YST.

结果

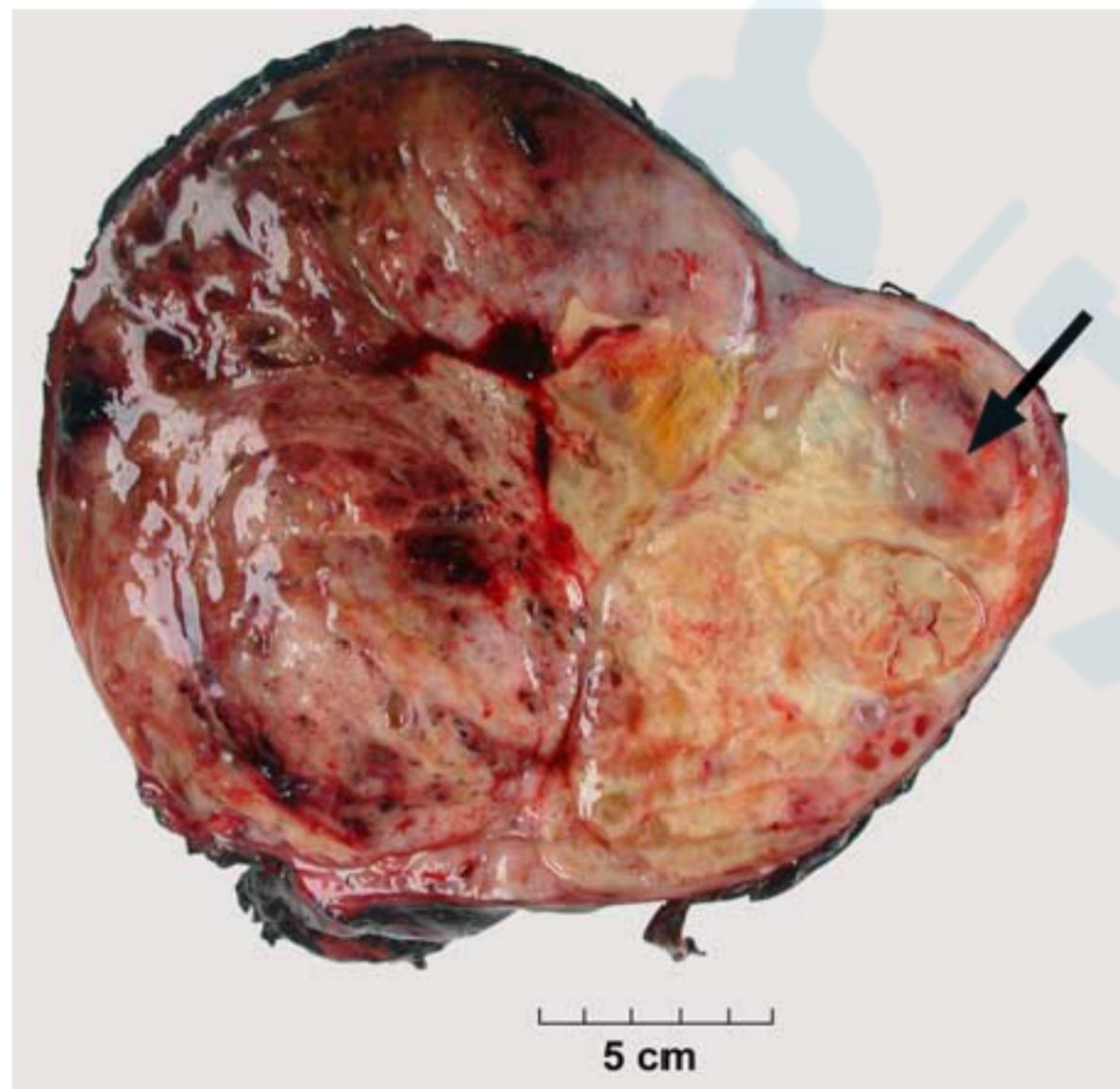
- 共检测来自11名患者的16例标本(腹膜后10例; 肺或纵隔3例; 腹腔内3例)
- 所有病例均为化疗后复发或转移病例(初次诊断睾丸GCT后4--132个月, 平均46.5个月)
- 2名患者在4--75个月内有数次SYST复发/转移, 对其7例标本分析12p在疾病过程中的变化
- 至随访截止时间, 6/11人在诊断SYST后7--112个月死亡, 平均39个月; 4例无进展生存, 随访时间1-74个月, 平均36个月; 1人在诊断SYST后39个月死于非相关疾病

形态特征

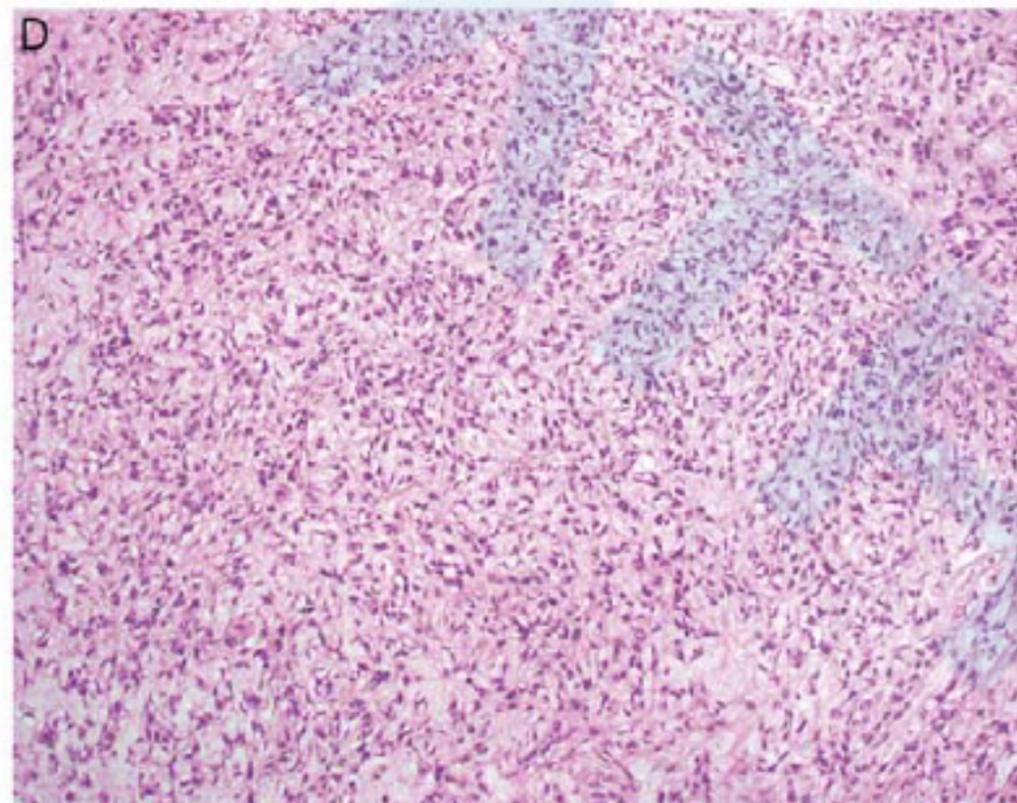
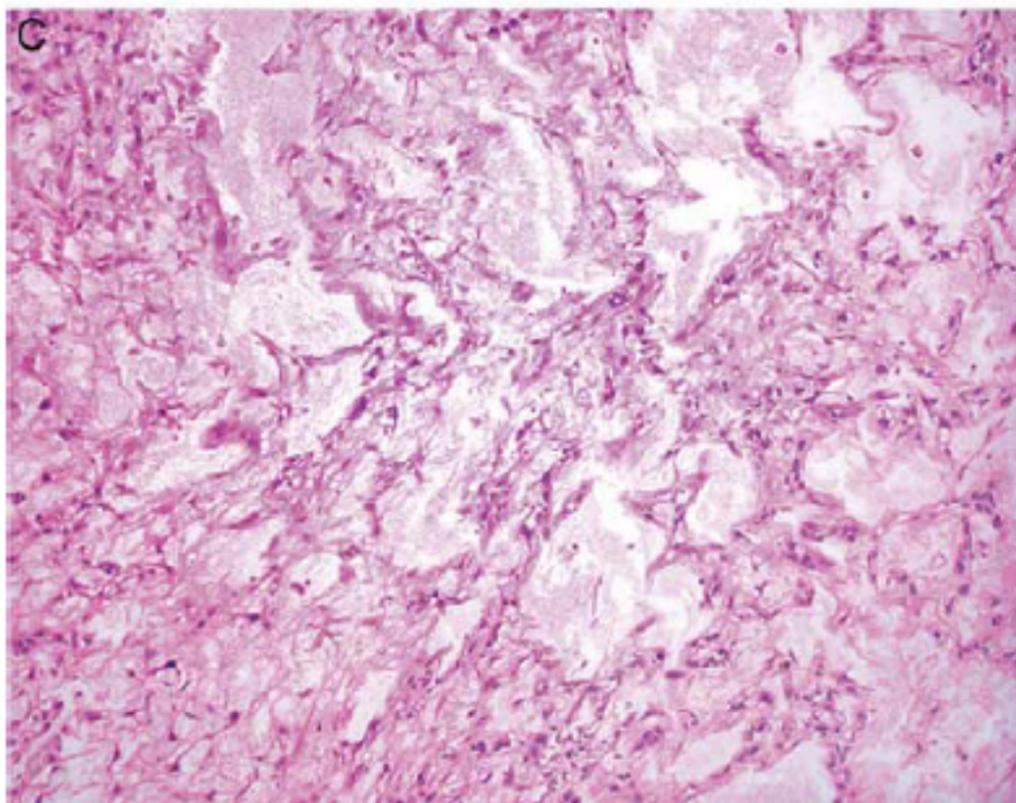
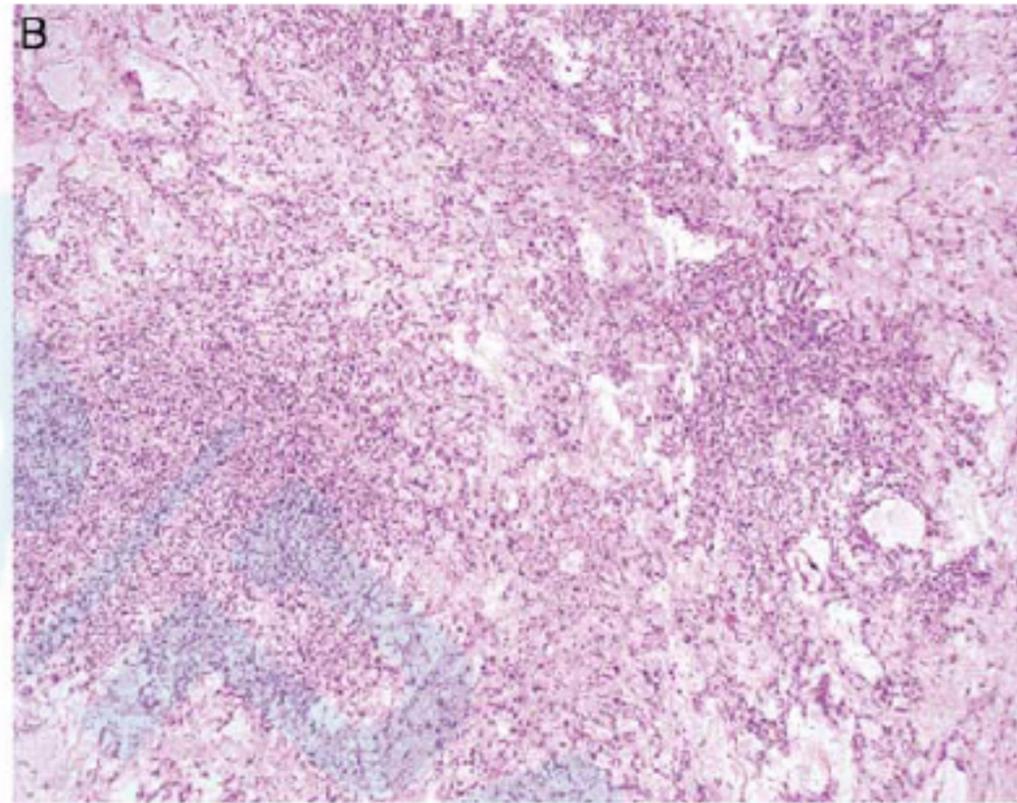
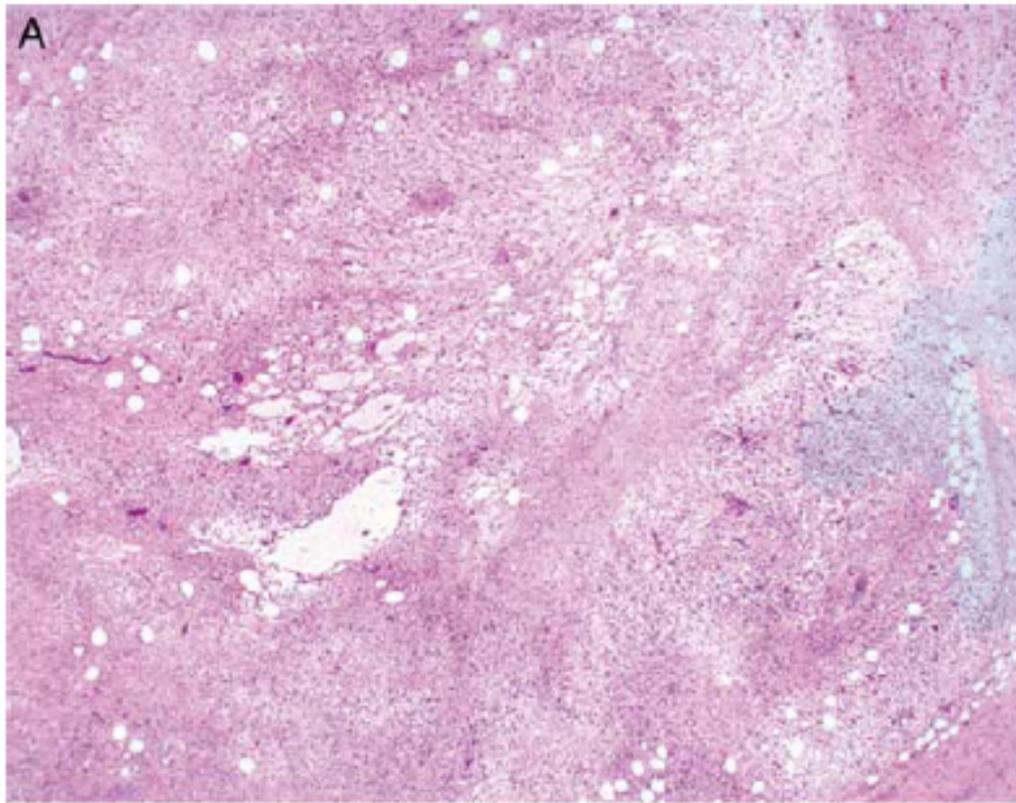
- 除1例患者外，均出现高级别肉瘤样形态
- 大多数肿瘤呈结节状或分叶状生长模式，梭形细胞与上皮样细胞混合存在，细胞低密度区与高密度区交替分布
- 所有肿瘤均为黏液样、纤维样和/或纤维黏液样间质，缺乏明显的束状排列模式。核异型性从轻度到重度不等

免疫组化

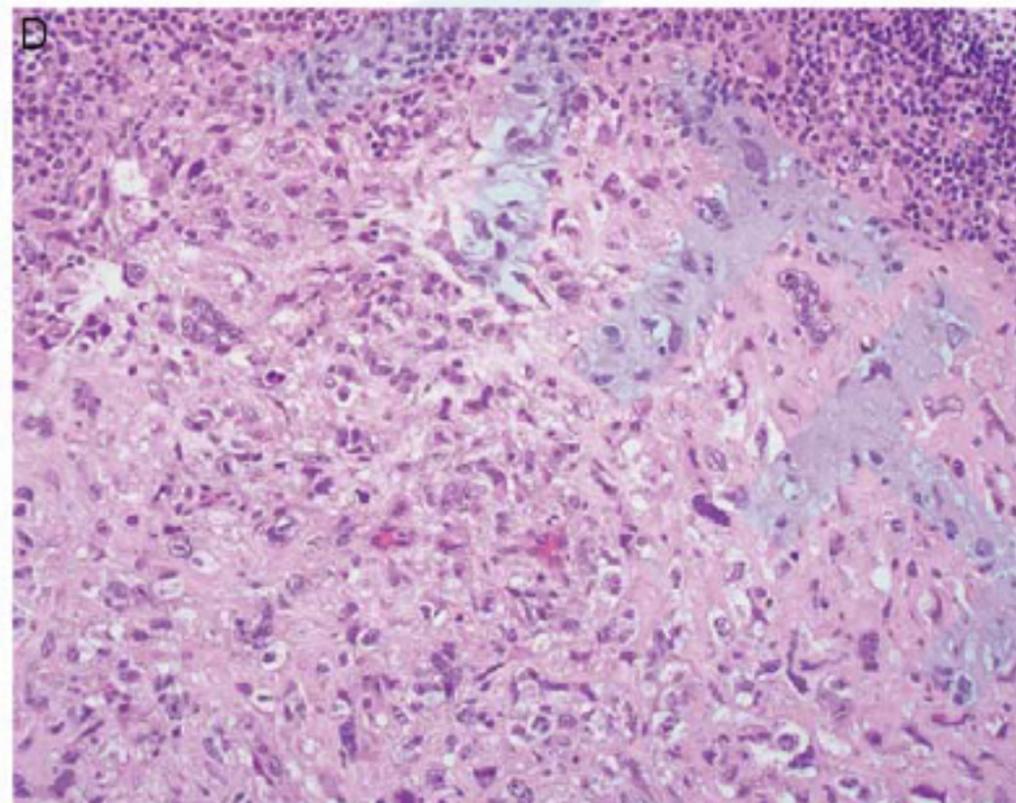
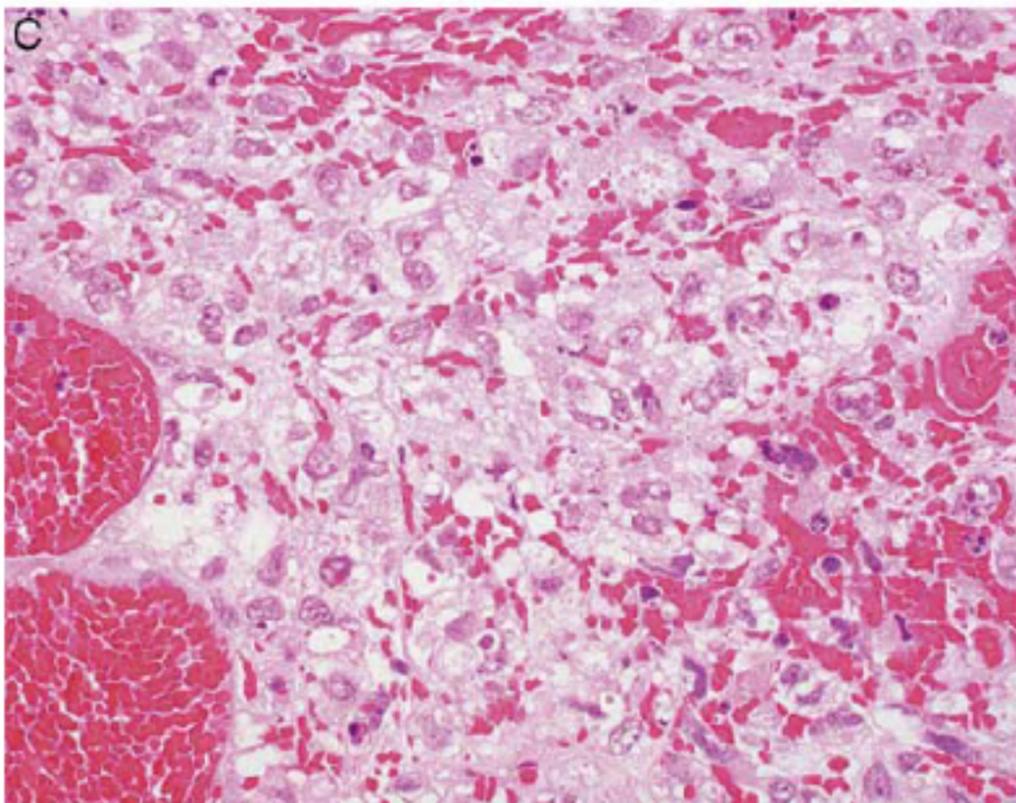
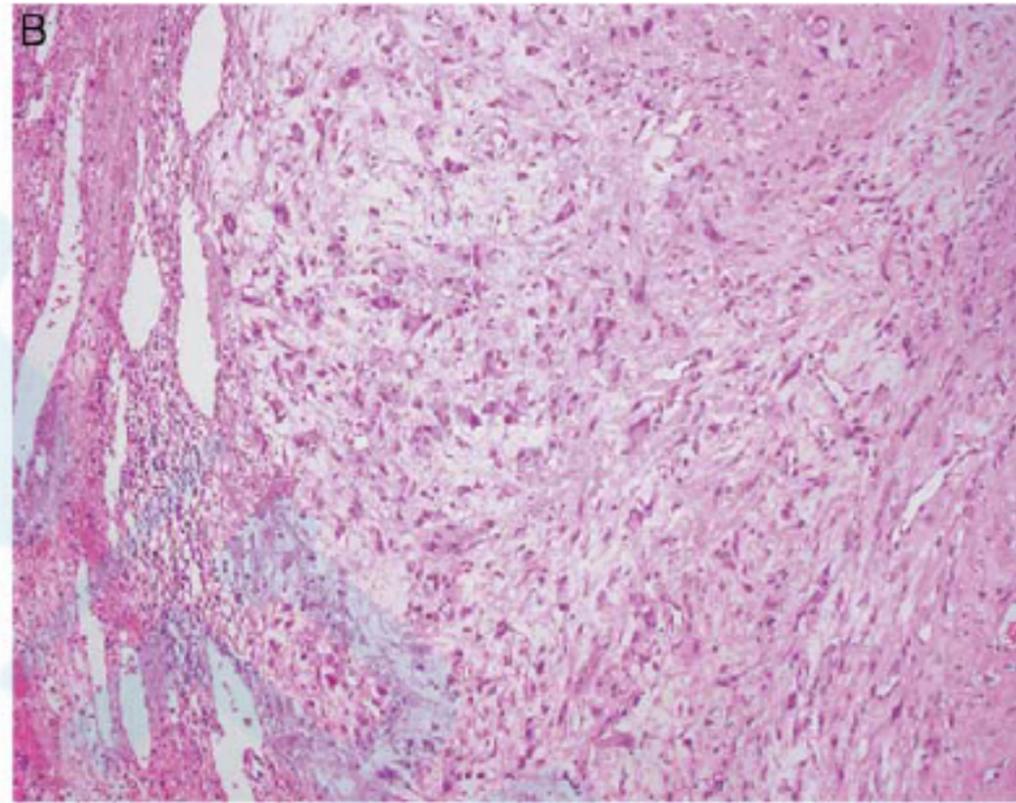
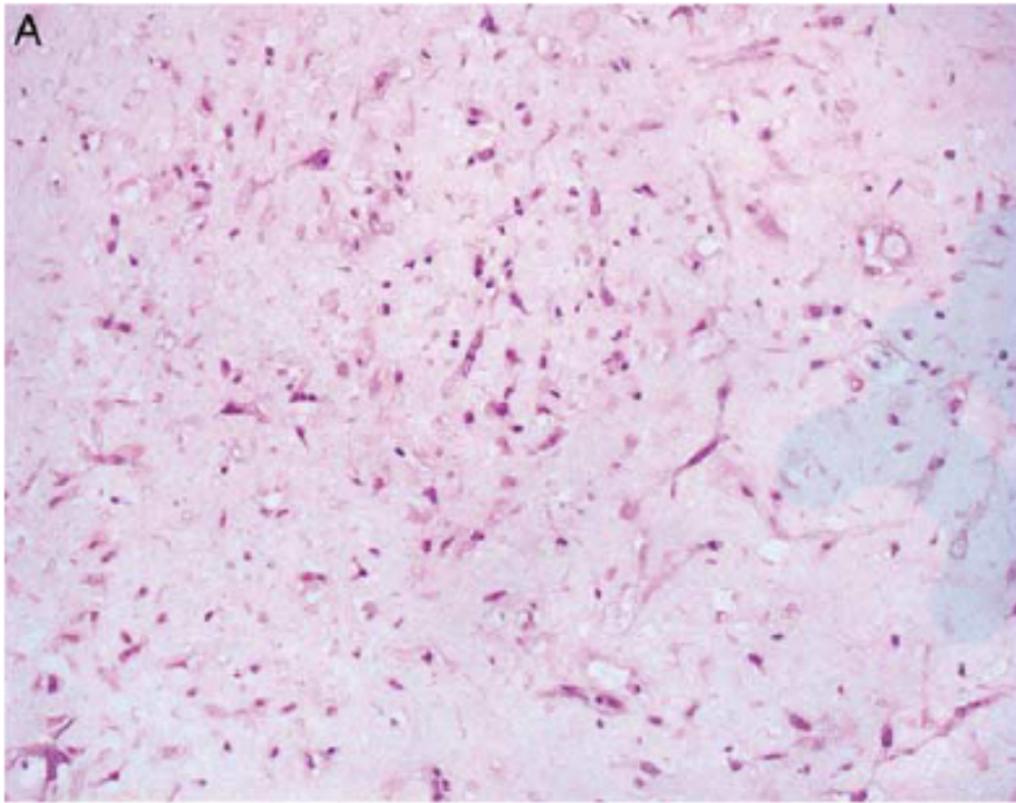
- 所有SYST均表达AE1/AE3和GPC3 (16/16)
- SALL4核阳性 (10/15) ， CD34局灶性阳性 (6/15) ， P63局灶性核阳性 (1/15)
- AFP、MUC4、GFAP、myogenin均阴性 (16/16)
- 约一半的SYST表现出平滑肌分化 (desmin, caldesmon, 和/或SMA表达)



- 大体表现为边界清楚的结节状肿块，切面褐色或灰白色，可见黏液样区域



- 分叶状生长，低密度区与高密度区交替分布，间质黏液样、纤维样和/或纤维黏液样



- 核异型性从轻度到重度不等
- 梭形细胞分布于黏液样间质中
- 瘤细胞呈高级别上皮样形态
- 细胞间可见基底膜样物质

FISH检测结果

- 16例标本中有13例检测到12P过表达；2例检测到12p缺失；1例出现12号染色体三体
- #4患者在初次诊断TGCT后78个月内有6次复发，对其第1、3、4和5次复发标本（分别发生于初次诊断后4、63、73和75个月）进行FISH检测，第1和第4次复发标本检测到12p缺失，而第3和第5次检测到12p过表达
- #1患者3次复发标本内均检测到12p持续的过表达
- 总之，11名患者中有10人在疾病过程中的某个点出现了12p的过表达，1人出现12号染色体三体

TABLE 2. Data Displaying 12p Overrepresentation in Sarcomatoid Yolk Sac Tumors

Patient #	Specimen #	12p: CEN12	i(12) (p10)	Comment/Inference
1	1	1.67	+	12p overrepresentation
	2	1.74	+	12p overrepresentation
	3	2.43	+	12p overrepresentation
2	4	1.72	+	12p overrepresentation
3	5	1.41	+	12p overrepresentation
4	6	1.44	+	12p overrepresentation
	7	0.7	-	12p loss
	8	0.82	-	12p loss
	9	1.51	+	12p overrepresentation
5	10	1.5	+	12p overrepresentation
6	11	1.36	+	12p overrepresentation
7	12	1.75	+	12p overrepresentation
8	13	1.39	+	12p overrepresentation
9	14	1.76	+	12p overrepresentation
10	15	1	-	Many cells with gain of chromosome 12
11	16	1.6	+	12p overrepresentation

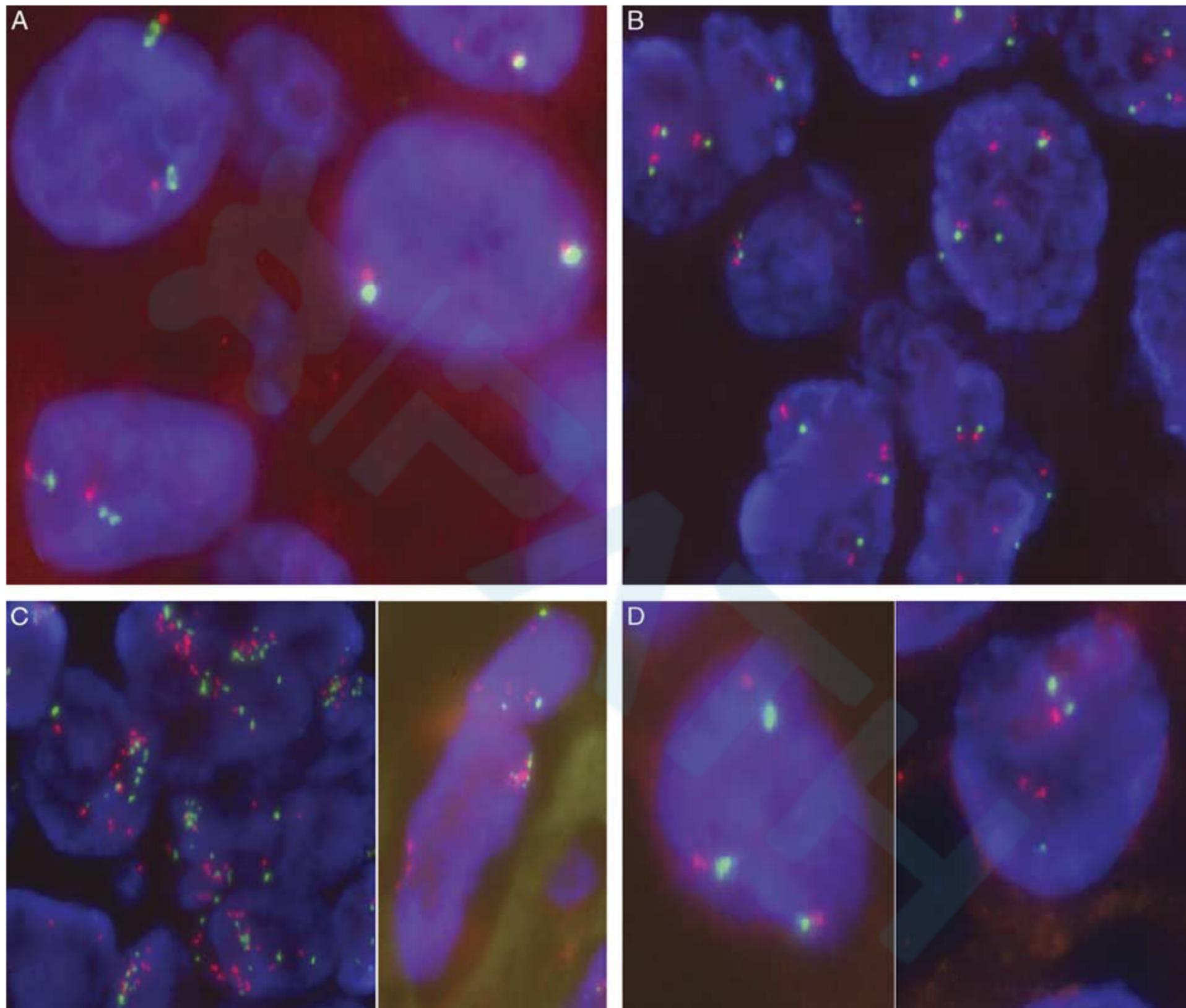


FIGURE 4. FISH analysis in sarcomatoid yolk sac tumor. A, Normal—2 red and 2 green signals. B, Possible multiple copies of iso12p—2 reds per 1 green. C, Overrepresentation of 12p characterized by increased red signals. D, Gain of entire chromosome 12, with 3 red and 3 green signals.

讨论与总结

- GCT引起的体细胞型恶性肿瘤并不罕见，尤其是在化疗后复发和转移病例中。最常见的组织学类型是横纹肌肉瘤和原始神经外胚层肿瘤，其次是腺癌和其他肉瘤。
- 过去认为其多数源于畸胎瘤的转化，而如今研究表明许多化疗后的TGCT患者发生肉瘤转化是SYSTs，通常发生在诊断TGCT数年后，血清AFP往往正常或轻微升高，表现出较高的侵袭性，化疗不敏感，多次复发/转移，预后差。

- 此类肿瘤通常呈结节状或分叶状生长模式，以梭形细胞为主或梭形细胞与上皮样细胞混合存在，细胞疏松区与致密区交替分布，缺乏明显的束状生长模式，间质黏液样、纤维样和/或纤维黏液样，约半数病例可见细胞间嗜酸性基底膜样物质。
- 所有病例均表达**AE1/AE3**和**GPC3**；部分病例局灶性表达SALL4、CD34、P63；约一半病例表现出平滑肌分化；不表达AFP、MUC4、GFAP、myogenin。
- 遗传学改变同其他GCTS，表现为12P改变，可通过间期核-FISH检测。除了12p过表达外，还可表现为整个12号染色体增加（12号染色体三体）。
- 对于在形态学上不明确的GCT衍生的恶性肿瘤，尤其在TGCTS晚期复发或病史不明的情况下12p的FISH检测有助于诊断。

感谢聆听