# SWI/SNF Chromatin-remodeling Complex Status in SMARCB1/INI1-preserved Epithelioid Sarcoma

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# 上皮样肉瘤 ES

- ES是一种独特的分类未明的肉瘤，在形态上主要由上皮样细胞构成。可能被误诊为良性病变，特别是良性肉芽肿性病变。

- 根据发病部位分为两种类型：

  经典型（远端型）；近端型（大细胞型）

- 免疫组化：

  Vim和上皮标记物(CK、EMA、Cam5.2等) +

  CD34 >50% +

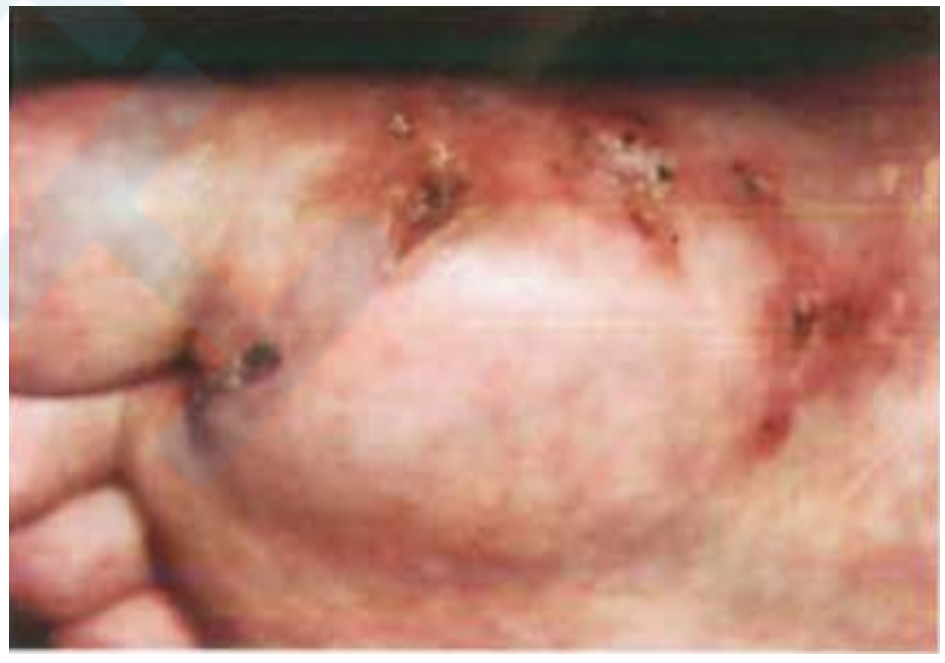
  INI-1常缺失

## □经典型（远端型）CES/DES

- 多发于10-40岁，中位年龄26岁，偶发于儿童和老年人，男女比2:1。
- 好发于四肢小关节旁，以手指、手、腕部和前臂的伸侧面，其次为膝、小腿胫前区、踝、足和趾。
- 位置表浅的ES，临床通常为生长缓慢的无痛性结节或斑块状缺损，呈孤立性或多发性，可有皮肤溃疡。表浅结节较小，通常<5cm。
- 深部软组织的ES，常附着于肌腱、腱鞘和/或腱膜相连。一般较大，可达15cm。
- 肉眼一般为小的、质硬、界限不清的真皮和/或皮下结节，或累及肌腱和/或筋膜的有不同程度坏死的较大肿物。

## □近端型（大细胞型）PES

- 多发于中青年，中位年龄35.5岁，较经典型年龄大，男性较女性多见。
- 好发于骨盆、会阴和生殖道，位置通常较深。



# 根据组织结构分为四个亚型：

## □经典型或普通型

结节状，细胞轻度异型，中央常见地图样坏死，坏死灶周边上皮样瘤细胞呈栅栏状排列，似肉芽肿样结构。结节周围较多增生的胶原纤维，可见横纹肌样细胞

## □血管瘤样亚型

瘤细胞结节中央有明显的出血囊性变，呈扩张的血管样

## □纤维瘤样亚型

肿瘤以梭形瘤细胞占优势

## □近端型

多结节状，瘤细胞黏附性差，异型性大，横纹肌样细胞丰富，常见坏死，但常无典型的肉芽肿样结构

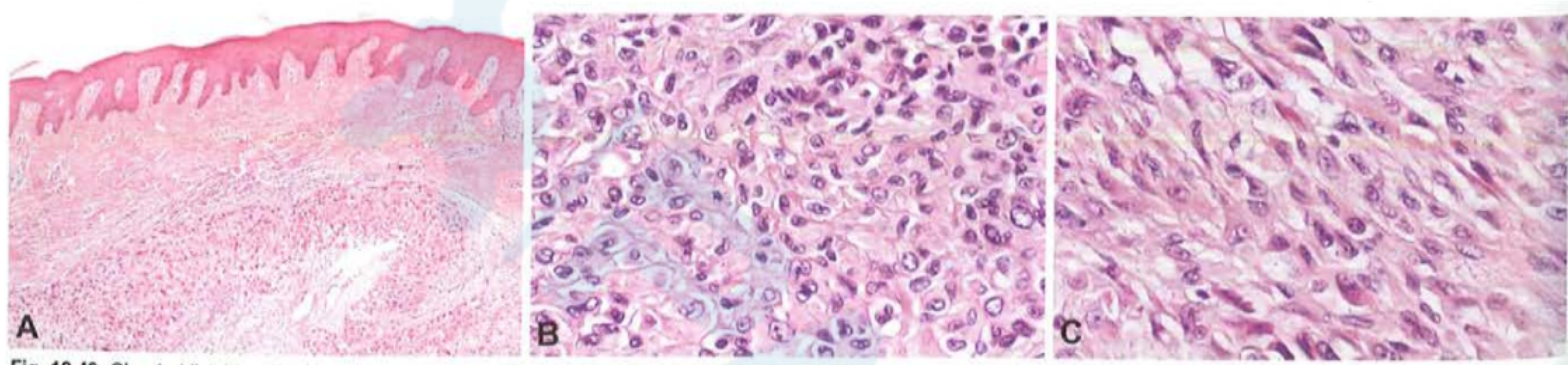



**Fig. 12.49** Classic (distal-type) epithelioid sarcoma. **A** Low-power magnification of a nodular lesion centred in the dermis composed of tumour cells surrounding an area of necrosis (pseudogranulomatous growth pattern). **B** Epithelioid tumour cells with abundant eosinophilic cytoplasm. **C** Cells with a plump spindle-shaped morphology.

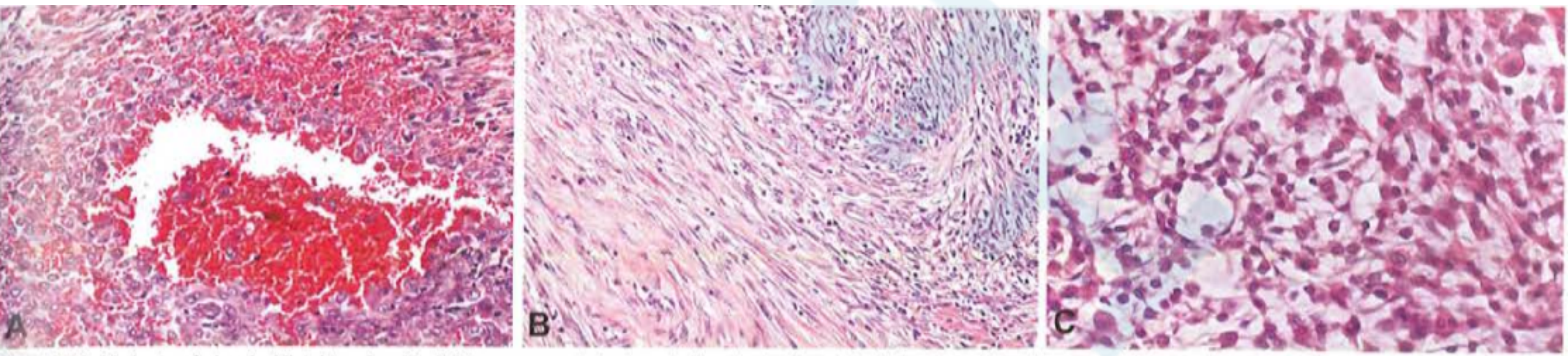



**Fig. 12.50** Variants of classic (distal-type) epithelioid sarcoma. **A** Angiomatoid variant with epithelioid cells surrounding a cystic space filled with blood. **B** Fibroma-like variant characterized by a fascicular or whorled arrangement of plump spindle-shaped cells. **C** Myxoid variant showing cord-like or reticular arrangement of epithelioid cells within a mucin-rich stroma.

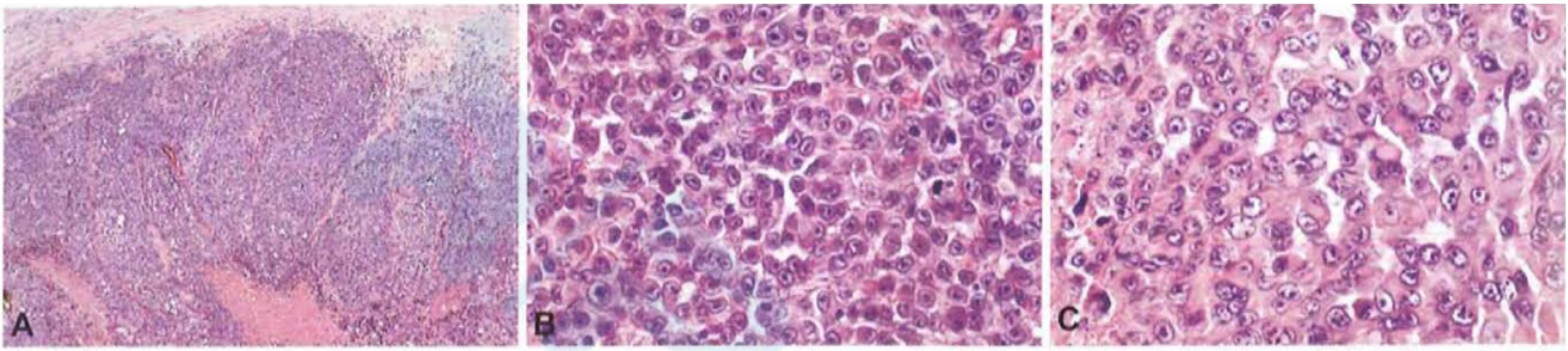



**Fig. 12.52** Proximal-type (large-cell) epithelioid sarcoma. **A** Low-power magnification shows a multinodular growth pattern of epithelioid cells with foci of tumour necrosis. **B** Pleomorphic epithelioid tumour cells with abundant, deeply eosinophilic cytoplasm and enlarged vesicular nuclei with prominent nucleoli. **C** Aggregates of rhabdoid cells with glassy intracytoplasmic hyaline inclusions and eccentrically located, vesicular nuclei.

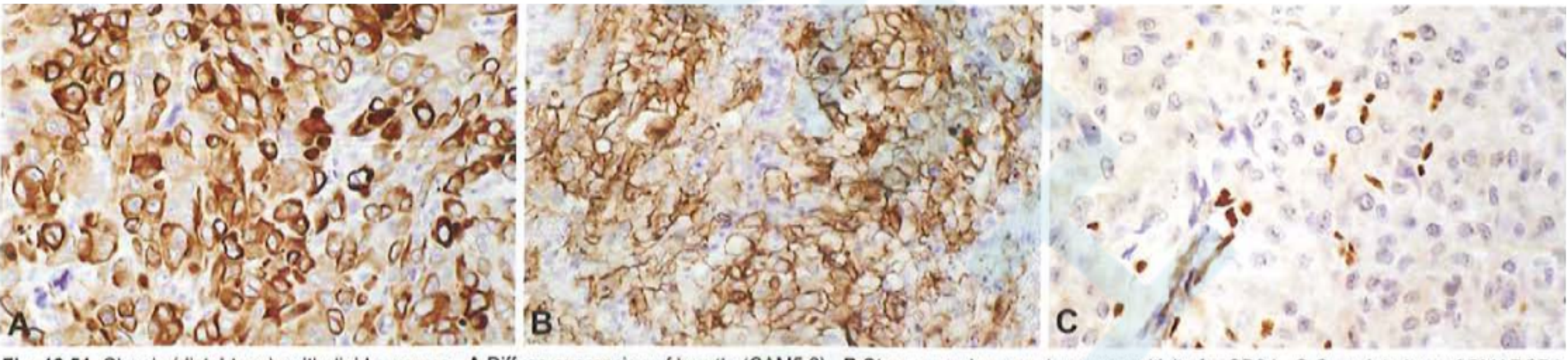



**Fig. 12.51** Classic (distal-type) epithelioid sarcoma. **A** Diffuse expression of keratin (CAM5.2). **B** Strong membranous immunopositivity for CD34. **C** Complete loss of SMARCB1 (INI1) protein expression in the tumour cells.

# 鉴别诊断

- 良性肉芽肿性病变
- 肾外恶性横纹肌样瘤
- 恶性黑色素瘤
- 上皮样血管肉瘤
- 滑膜肉瘤
- 鳞状细胞癌

# SWI/SNF

- SWI/SNF复合物是ATP依赖性染色体重塑复合物家族成员，可以利用ATP水解产生的能量促使染色体构象发生改变，使转录因子易于接近核小体DNA，从而对基因转录进行调控。
- 最初在酵母中发现的 SWI/SNF 复合物调节同源转换核酸内切酶（HO）和蔗糖转化酶（SUC2）的表达。在这些复合物中，HO是开关（SWI），SUC2是蔗糖非发酵（SNF）所必需的。
- SWI/SNF复合物在一系列重要生物行为如分化，增生及DNA损伤中发挥重要作用。

# SWI/SNF

- SWI/SNF复合物分两个亚类：一是BAF复合物，另一类是PBAF复合物。
- SWI/SNF复合物由三类亚基构成：

  - 高度保守的核心亚基（核心亚基主要参与双链断裂(DSB)修复和核苷酸切除修复）

    ATP酶性亚基 —— BRG1(SMARCA4)/BRM(SMARCA2); BRG1(SMARCA4)

    SWI3 —— BAF155(SMARCC1) 及 BAF170(SMARCC2)

    SNF5 —— INI1/ SNF5/BAF47(SMARCB1)

  - 功能特异性的特征亚基（与延伸和转录相关）

    PBRM1(BAF180) , ARID1A(BAF250A) 等

  - 辅助亚基（与增殖和分化相关）

    BAF57 , BAF53A/B , BAF60A/B/C 和 β -actin 等

SWI/SNF Family

SWI/SNF-typeA

BAF complex

Core subunit

ATPase: BRM or BRG1 (*SMARCA2/SMARCA4*)

SWI3:
- BAF155 (*SMARCC1*)
- BAF170 (*SMARCC2*)

SNF5: hSNF5/BAF47/INI1 (*SMARCB1*)

DSB repair and nucleotide excision repair

Signature subunit
- BAF250A (*ARID1A*)
- BAF250B (*ARID1B*)

Elongation, transcription

Accessory subunit
- BAF60A/B/C (*SMARCD1/2/3*)
- BAF57 (*SMARCE1*)
- BAF53A/B (*ACTL6A/B*)
- Actin: β-actin

Proliferation, differentiation

SWI/SNF-typeB

PBAF complex

Core subunit

ATPase: BRG1 (*SMARCA4*)

SWI3:
- BAF155 (*SMARCC1*)
- BAF170 (*SMARCC2*)

SNF5: hSNF5/BAF47/INI1 (*SMARCB1*)

DSB repair and nucleotide excision repair

Signature subunit
- BAF180 (*PBRM1*)
- BAF200B (*ARID2*)
- BRD7

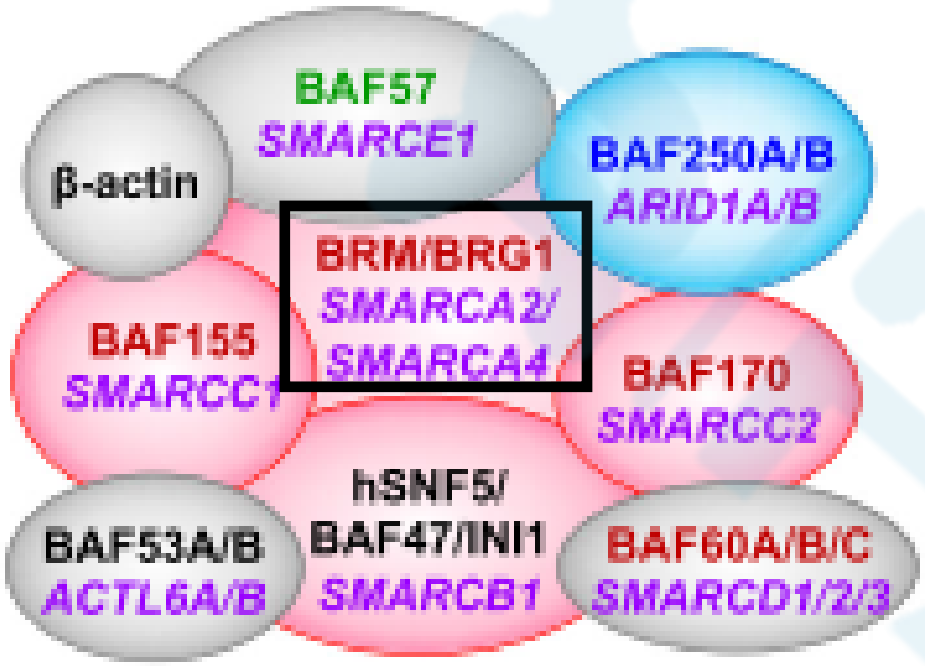
Elongation, transription

Accessory subunit
- BAF60A/B/C (*SMARCD1/2/3*)
- BAF57 (*SMARCE1*)
- BAF53A/B (*ACTL6A/B*)
- Actin: β-actin

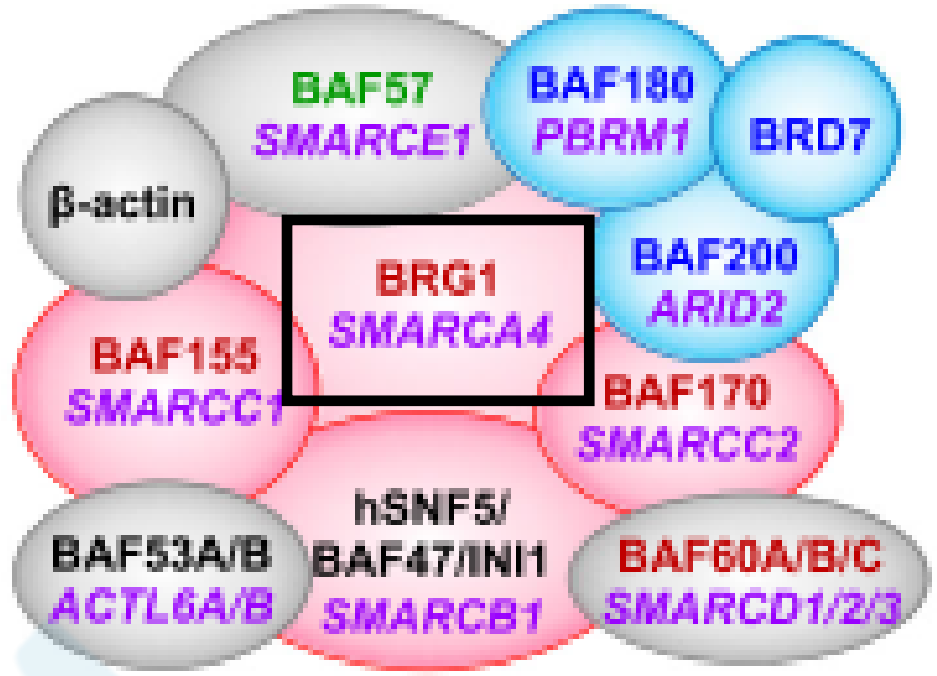
Proliferation, differentiation

Composition

Function




BAF complex




PBAF complex

- Core subunits
- Signature subunits
- Accessory subunits

DSB repair and nucleotide excision repair

Elongation and transcription

Cell-cycle, proliferation, differentiation and stem cell self-renewal

# INI1

- INI1位于染色体**22q11.2**，属于肿瘤抑制基因。
- INI1缺失首先在肾及肾外恶性横纹肌样瘤、非典型畸胎样/横纹肌样瘤（AT/RT）中发现，并成为其特征性的诊断指标。
- 随后INI1失表达在不同肿瘤中发现：ES，上皮样恶性神经鞘瘤，肾髓质样癌等。
- **经典型 ES 81%-93%** 病例缺失，**近端型 ES 76%-95%** 病例缺失。
- 新近研究指出：位于**19p13.2**的BRG- 1基因无义突变亦可导致染色体重塑复合物发生变化，而位于**22q11.2**的INI1基因保留，即INI1阳性。BRG- 1蛋白缺失是INI1表达阳性的AT/RT的标记。
- 但是，目前没有关于INI1保留的ES病例中的SWI / SNF染色体重塑复合物的研究。

# MATERIALS AND METHODS

- between 1980 and 2015
- the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
- selected from among > 20,000 cases of bone and soft-tissue tumors
- Sixty epithelioid sarcoma

  proximal-type, 29 cases

  conventional-type, 31 cases
- Immunoreactivity：

  −, no staining

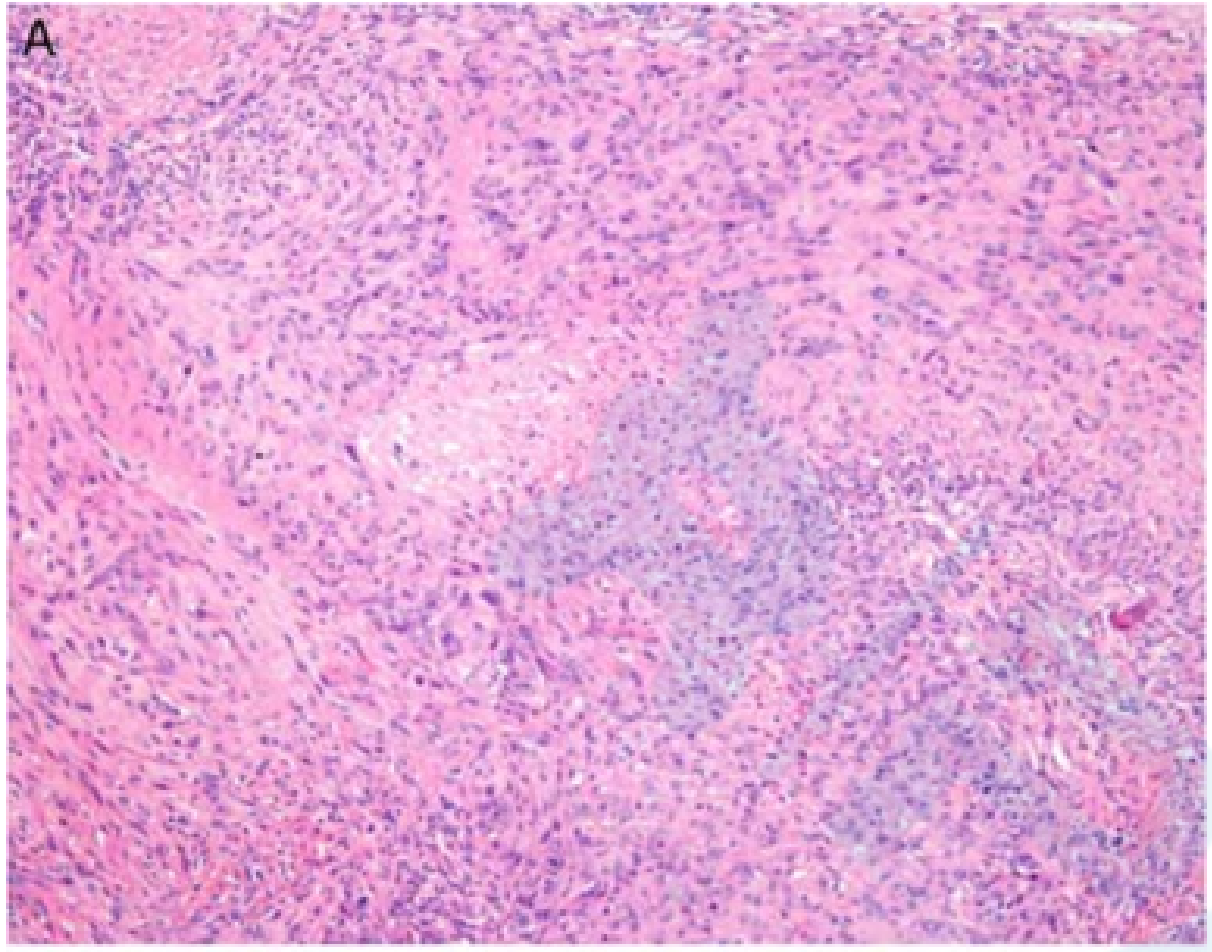
  focal+, <25% of tumor cells reactive

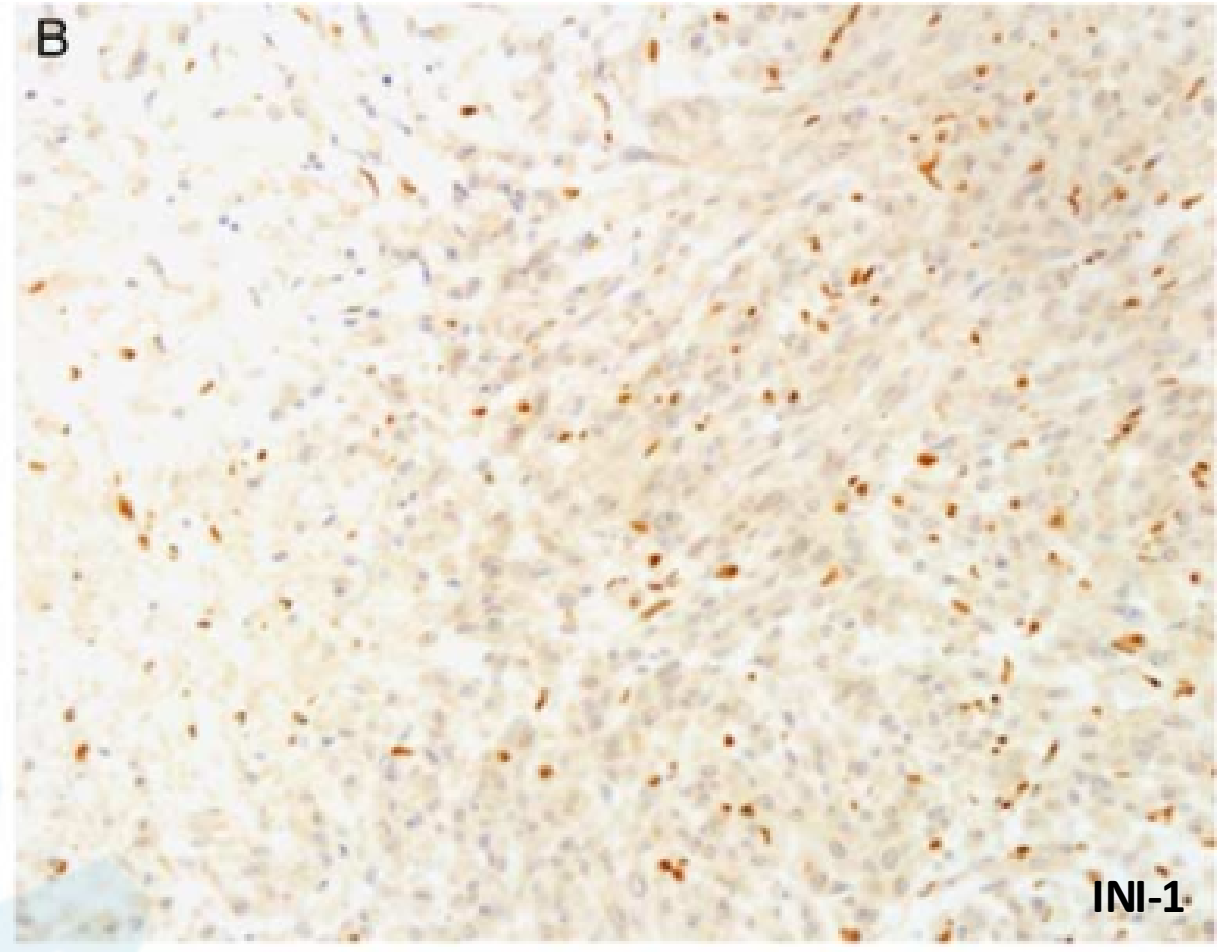
  +, >25% of tumor cells reactive

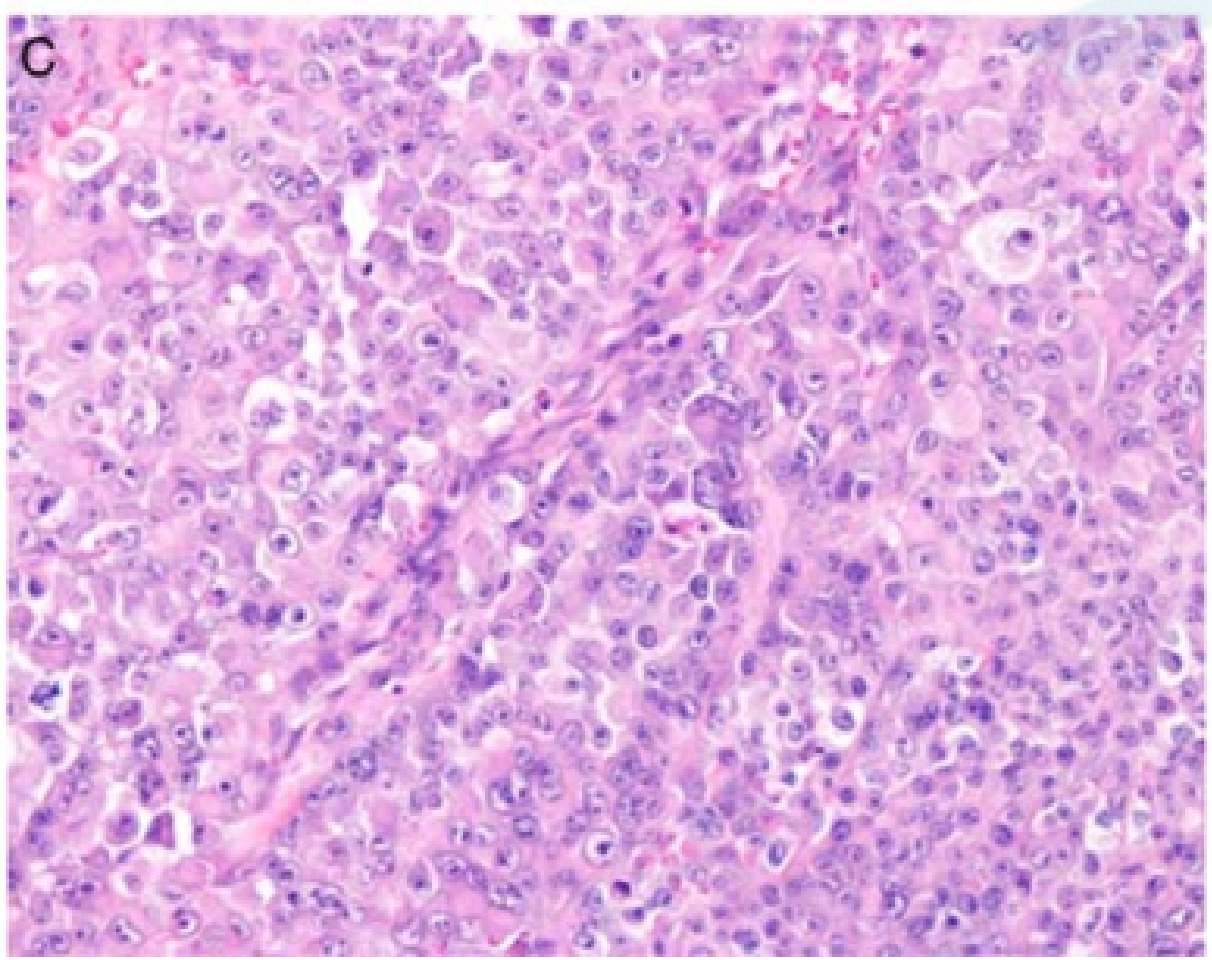
# MATERIALS AND METHODS

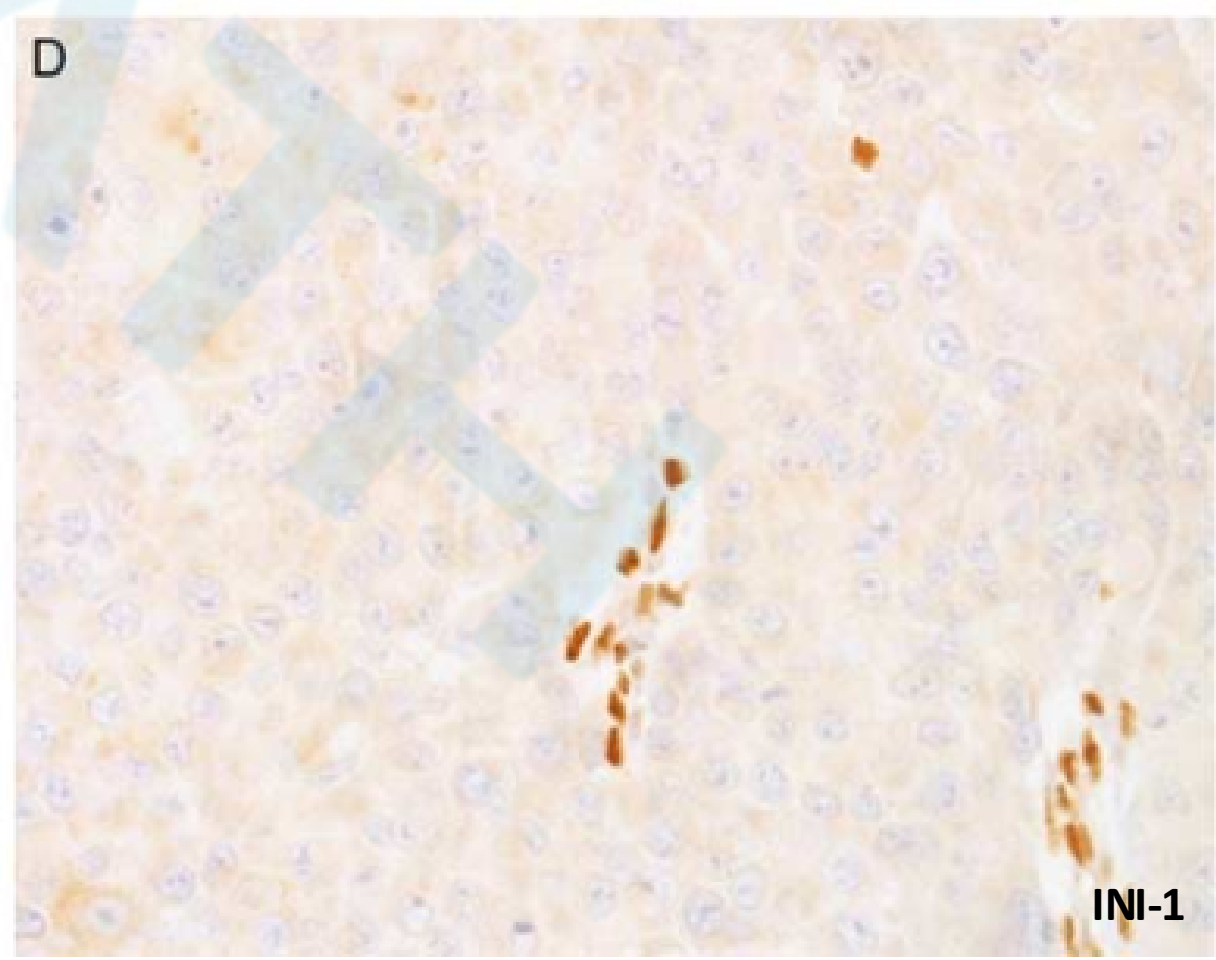
**TABLE 1.** Primary Antibodies Used in This Study

| Antigen | Clone | Source | Dilution | Antigen Retrieval Time |
|---|---|---|---|---|
| SMARCB1/INI1 | 25/BAF47 | BD Transduction Laboratories, San Diego, CA | 1:250 | Target Retrieval Solution, 99°C, 20 min |
| SMARCA4/BRG1 | G-7 | Santa Cruz Biotechnology, Dallas, TX | 1:25 | Target Retrieval Solution, 110°C, 15 min |
| SMARCC1/BAF155 | DXD7 | Santa Cruz Biotechnology | 1:50 | Target Retrieval Solution, 110°C, 15 min |
| SMARCC2/BAF170 | E-6 | Santa Cruz Biotechnology | 1:100 | Target Retrieval Solution, 110°C, 15 min |
| ARID1A | Polyclonal | Sigma-Aldrich, St Louis, MO | 1:500 | Target Retrieval Solution, 99°C, 40 min |
| CAM5.2 | CAM5.2 | Becton-Dickinson, Mountain View, CA | 1:20 | Trypsin, 37°C, 30 min |
| AE1/AE3 | AE1/AE3 | Dako Cytomation, Carpinteria, CA | 1:1000 | Citrate buffer, 99°C, 30 min |
| Glypican-3 | 1G12 | BioMosaics, Burlington, VT | 1:200 | Target Retrieval Solution, 99°C, 20 min |
| SALL4 | 6E3 | Abnova, Taipei, Taiwan | 1:1000 | Citrate buffer, 99°C, 20 min |
| ERG | 9FY | BioCareMedical, Concord, CA | 1:100 | Target Retrieval Solution, 99°C, 20 min |


A


B
INI-1


C


D
INI-1

# RESULTS

**TABLE 2.** Clinicopathologic Features in SMARCB1/INI1-preserved Epithelioid Sarcoma

| Case | Age (y) | Sex | Site | Size (cm) | Rhabdoid Cell | Mitotic Count | Tumor Necrosis | Prognosis |
|---|---|---|---|---|---|---|---|---|
| Proximal type | | | | | | | | |
| P-1 | 76 | M | Chest wall | 15 | − | 3 | 1 | 12 mo DOD |
| P-2 | 57 | M | Abdominal wall | 15 | + | 3 | 1 | 2 mo DOD |
| P-3 | 15 | M | Thigh | 10 | + | 3 | 2 | 6 mo DOD |
| P-4 | 74 | M | Thigh | 15 | + | 3 | 2 | 3 mo DOD |
| P-5 | 41 | M | Axilla | 10 | + | 3 | 2 | 4 mo DOD |
| P-6 | 65 | M | Forearm | 7.0 | + | 2 | 2 | 3 mo DOD |
| Conventional type | | | | | | | | |
| C-1 | 8 | M | Lower leg | 2.5 | + | 1 | 1 | 155 mo NED |
| C-2 | 33 | M | Hand | 1.3 | − | 1 | 0 | NA |

DOD indicates dead of disease; M, male; NA, not available; NED, no evidence of disease.

52例 INI1 缺失，8例 INI1 保留——2例经典型；6例近端型

诊断年龄8-76岁，中位年龄49岁

5例位于四肢（2例上肢和3例下肢），3例躯干

最大直径为1.3-15 cm， 中位10 cm

6例出现了横纹肌样细胞

**Table 1.1** Definition of histopathological parameters in the FNCLCC grading system

| Histological parameter | Definition |
|---|---|
| **Tumour differentiation** (see Table 1.2) | • Score 1: Sarcomas closely resembling normal adult mesenchymal tissue and potentially difficult to distinguish from the counterpart benign tumour (e.g. well-differentiated liposarcoma, well-differentiated leiomyosarcoma)<br>• Score 2: Sarcomas for which histological typing is certain (e.g. myxoid liposarcoma, myxofibrosarcoma)<br>• Score 3: Embryonal and undifferentiated sarcomas, synovial sarcomas, sarcomas of doubtful type |
| **Mitotic count** (established on the basis of 10 HPF; 1 HPF measures 0.1734 $mm^2$) | • Score 1: 0–9 mitoses per 10 HPF<br>• Score 2: 10–19 mitoses per 10 HPF<br>• Score 3: > 19 mitoses per 10 HPF |
| **Tumour necrosis** | • Score 0: no necrosis<br>• Score 1: < 50% tumour necrosis<br>• Score 2: ≥ 50% tumour necrosis |
| **Histological grade** | • Grade 1: total score 2, 3<br>• Grade 2: total score 4, 5<br>• Grade 3: total score 6, 7, 8 |

FNCLCC, Fédération Nationale des Centres de Lutte Contre le Cancer; HPF, high-power field

Modified from {494}

# RESULTS

**TABLE 2.** Clinicopathologic Features in SMARCB1/INI1-preserved Epithelioid Sarcoma

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| P-6 | 65 | M | Forearm | 7.0 | + | 2 | 2 | 3 mo DOD |
| Conventional type | | | | | | | | |
| C-1 | 8 | M | Lower leg | 2.5 | + | 1 | 1 | 155 mo NED |
| C-2 | 33 | M | Hand | 1.3 | − | 1 | 0 | NA |

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近端型ES有丝分裂指数和坏死评分明显高于经典型 ES

# RESULTS

**TABLE 2.** Clinicopathologic Features in SMARCB1/INI1-preserved Epithelioid Sarcoma

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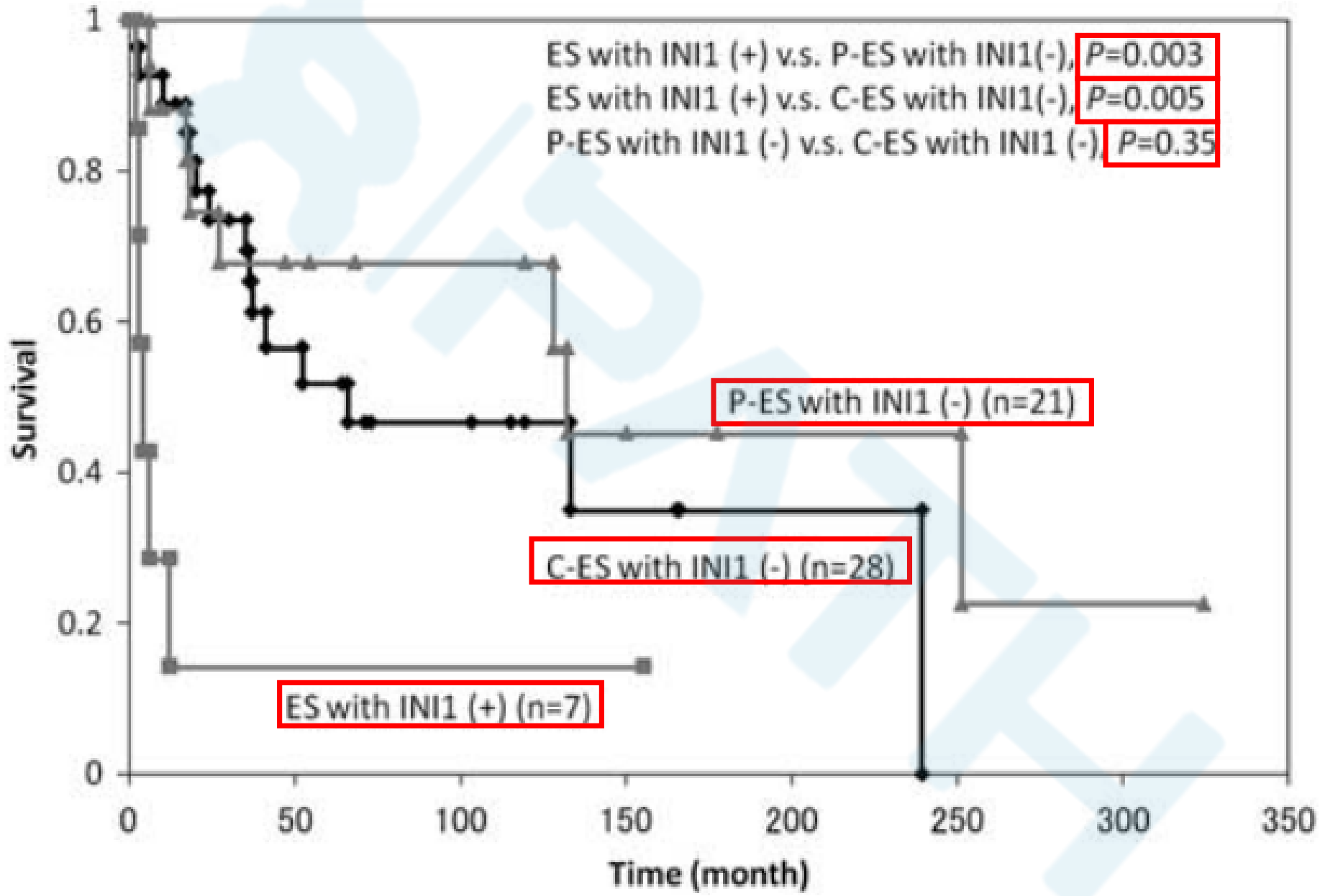
| C-1 | 8 | M | Lower leg | 2.5 | + | 1 | 1 | 155 mo NED |
| C-2 | 33 | M | Hand | 1.3 | − | 1 | 0 | NA |

DOD indicates dead of disease; M, male; NA, not available; NED, no evidence of disease.

随访3-155 m（平均4 m），所有近端型的患者都死于疾病，而1例经典型患者无病生存

# RESULTS


ES with INI1 (+) v.s. P-ES with INI1(-), P=0.003
ES with INI1 (+) v.s. C-ES with INI1(-), P=0.005
P-ES with INI1 (-) v.s. C-ES with INI1 (-), P=0.35
P-ES with INI1 (-) (n=21)
C-ES with INI1 (-) (n=28)
ES with INI1 (+) (n=7)
Survival
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300
350
Time (month)

# RESULTS

**TABLE 3.** Comparison of Overall Survival Between the Various 2 Groups

| Group | No. Cases | *P* |
|---|---|---|
| SMARCB1/INI1-deficient epithelioid sarcoma of ≥5 cm in size | 23 | 0.00006 |
| SMARCB1/INI1-preserved epithelioid sarcoma of ≥5 cm in size | 6 | |
| SMARCB1/INI1-deficient proximal-type epithelioid sarcoma of ≥5 cm in size | 9 | 0.001 |
| SMARCB1/INI1-preserved proximal-type epithelioid sarcoma of ≥5 cm in size | 6 | |
| SMARCB1/INI1-deficient epithelioid sarcoma of ≥7 cm in size | 13 | 0.0006 |
| SMARCB1/INI1-preserved epithelioid sarcoma of ≥7 cm in size | 6 | |
| SMARCB1/INI1-deficient proximal-type epithelioid sarcoma of ≥7 cm in size | 5 | 0.01 |
| SMARCB1/INI1-preserved proximal-type epithelioid sarcoma of ≥7 cm in size | 6 | |

无论肿瘤的大小或分型，INI1 + ES 患者预后更差

# RESULTS

**TABLE 4.** Immunohistochemical Profiles in SMARCB1/INI1-preserved Epithelioid Sarcoma

| Case | SMARCB1 | SMARCA4 | SMARCC1 | SMARCC2 | ARID1A | CAM5.2 | AE1/AE3 | GPC3 | SALL4 | ERG |
|---|---|---|---|---|---|---|---|---|---|---|
| Proximal type | INI1 | BRG1 | BAF155 | BAF170 | | | | | | |
| P-1 | + | + | + | − | + | Focal+ | + | − | − | − |
| P-2 | + | + | + | + | + | − | Focal+ | − | − | − |
| P-3 | + | + | + | + | + | Focal+ | Focal+ | Focal+ | − | − |
| P-4 | + | − | + | + | + | Focal+ | Focal+ | − | − | − |
| P-5 | + | − | + | + | + | + | + | − | − | − |
| P-6 | + | + (mosaic) | − | + | + | + | + | − | − | − |

8例 INI1 保留的病例有6例可以做进一步的免疫组化分析

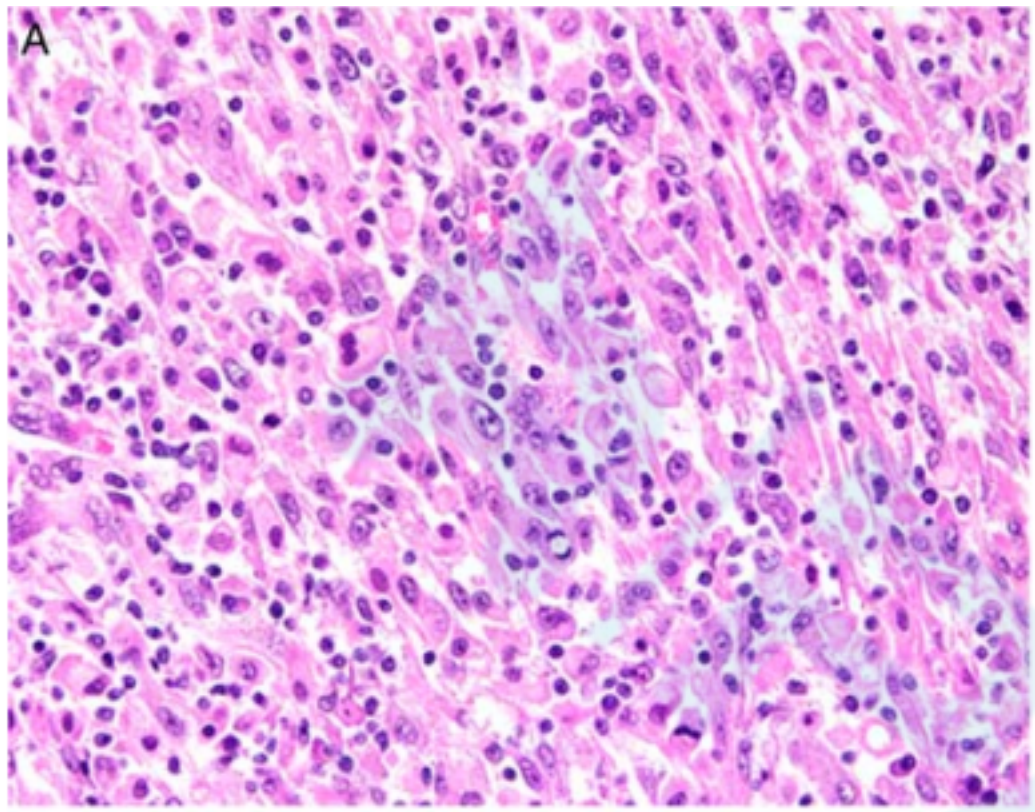
1例 BAF170 完全缺失

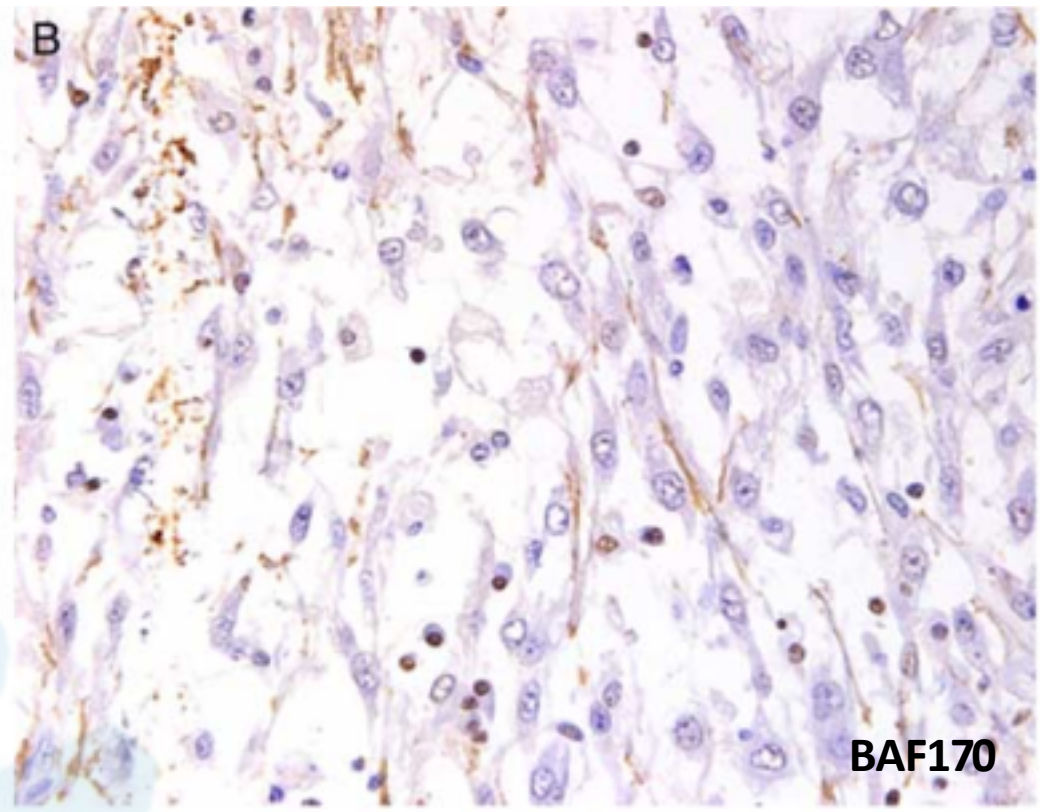
2例 SWI/SNF 复合物的这5种亚基均保留

2例 BRG1 完全缺失

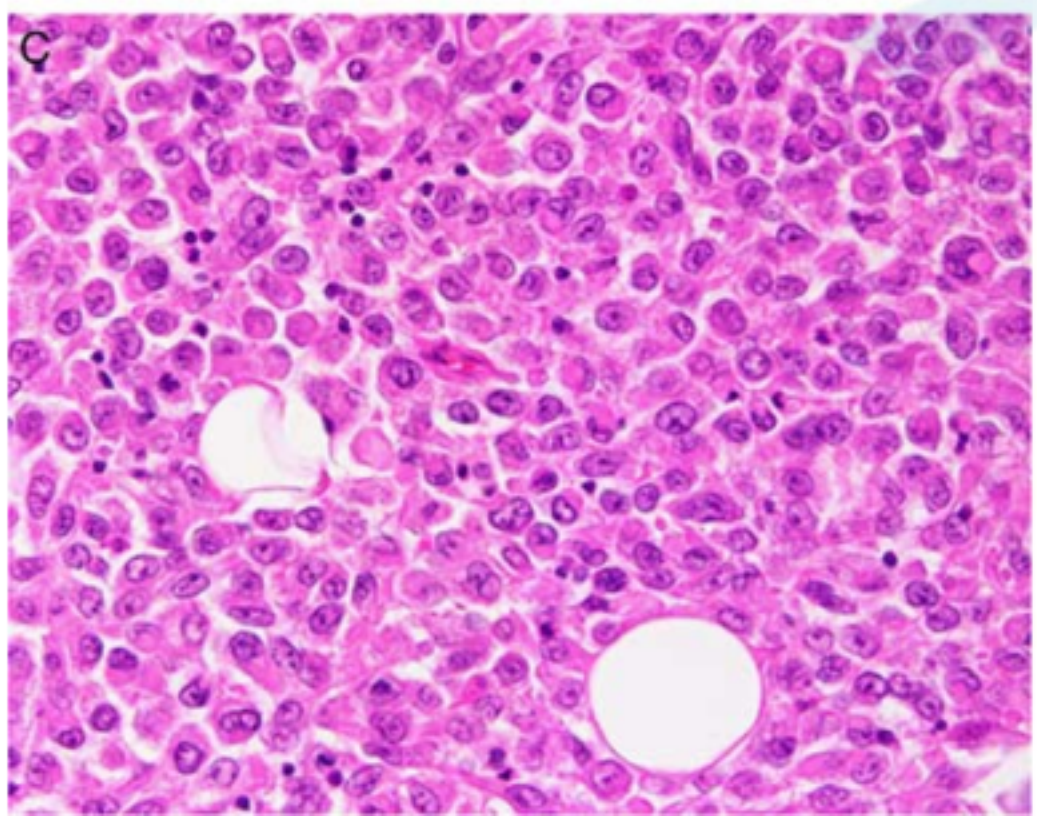
1例 BRG1 马赛克式保留，BAF155完全缺失

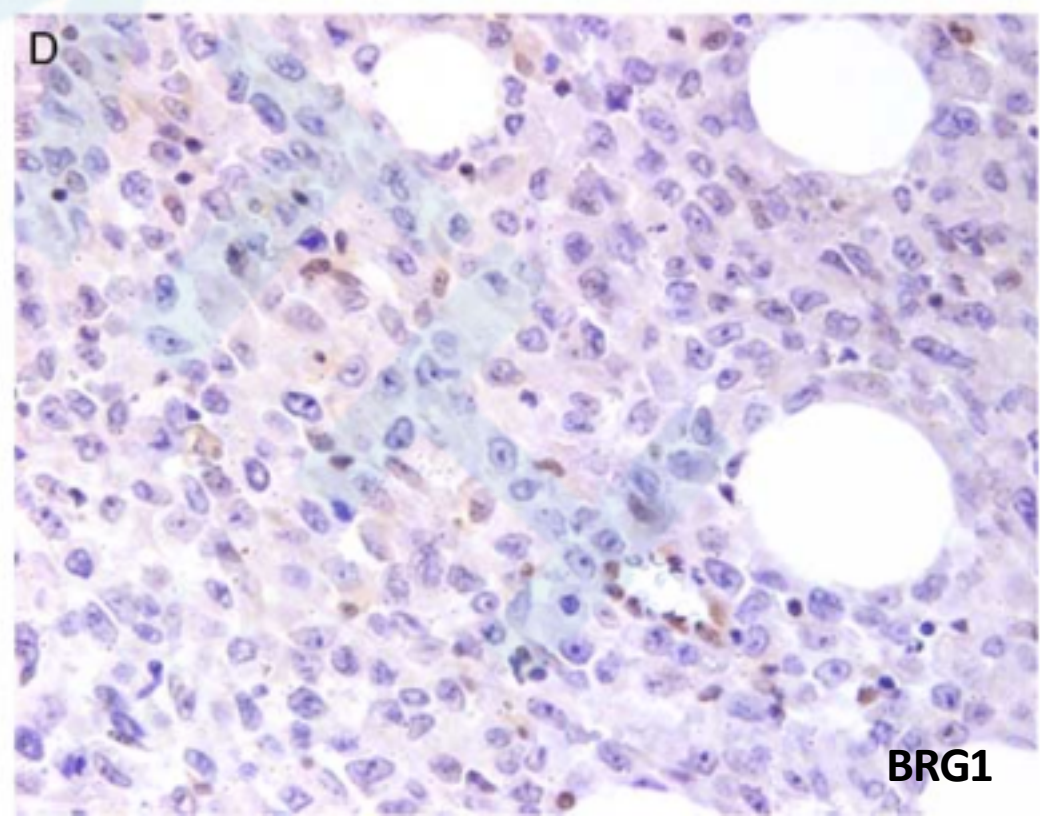
P-1



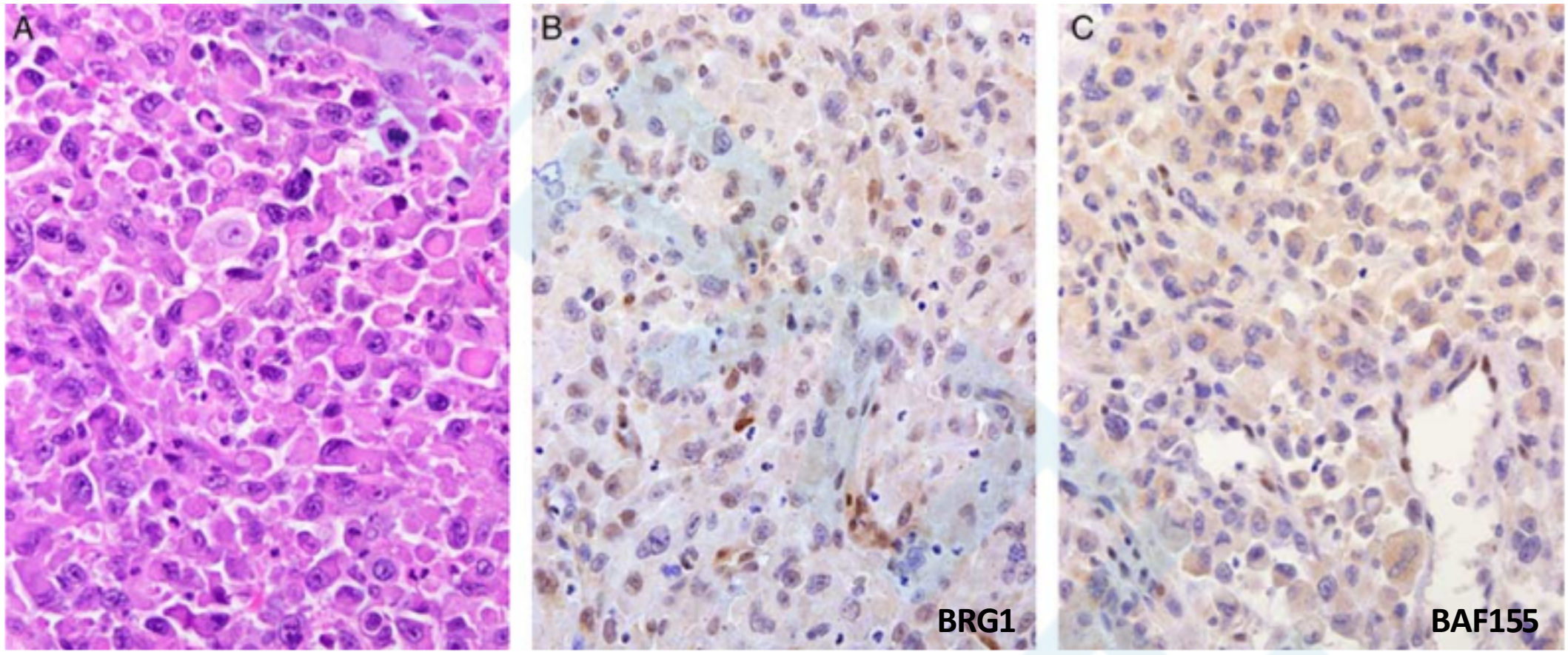

P-5



# RESULTS

P-6




1例 BRG1 马赛克式保留，BAF155完全缺失

# DISCUSSION

- 在除2例近端型病例保留SWI/SNF复合物的核心亚基蛋白，其余病例均显示至少一种核心亚基蛋白的完全缺失，这提示SWI/SNF复合物功能障碍可能与肿瘤的发生或去分化有关。

- 在INI1缺失的恶性间叶肿瘤中偶见横纹肌样特征。最近，据报道具有横纹肌样特征的上皮样癌表现出SWI/SNF复合物的核心亚基蛋白的异常表达。 而在本研究中，这些INI1保留的上皮样肉瘤异常表达其复合物的其他亚基，也可见横纹肌样特征。 因此，SWI/SNF相关蛋白的缺乏可能与横纹肌样细胞形态相关。

- 相反，本组研究中有2例近端型上皮样肉瘤显示了SWI/SNF复合物的核心亚基蛋白的完整表达。 这很可能是由SWI/SNF复合物的其他组分或SWI/SNF转录后调节机制的改变所驱动的。为了阐明SWI/SNF复合物完整表达的上皮样肉瘤的肿瘤发生的原因，还需要大量病例的进一步研究。

# DISCUSSION

- 在本研究中，发现 INI1+ ES 的生物学行为比 INI1- ES 更具侵袭性。因此，INI1蛋白的保留可能是上皮样肉瘤，尤其是近端型上皮样肉瘤，有用的不利预后因素。

- SWI/SNF复合物INI1保留而其他亚基缺失，可能对其复合物的功能障碍产生强烈影响。

- 虽然报道的近端型上皮样肉瘤预后比经典型上皮样肉瘤差，但在本研究中他们没有显著的统计学差异，这很可能是病例少的缘故。为了阐明SWI/SNF复合物核心亚基蛋白的缺失对预后和功能的影响，还需要大量病例的进一步研究。

# DISCUSSION

- BRG1- TS 预后极差，中位生存期为7个月

  应该与 ES 鉴别，尤其是INI1+ BRG1- ES

| | AE1 / AE3 | CD34 | SOX2 | p53 | SALL4 |
|---|---|---|---|---|---|
| BRG1- TS | 6/12 (50 %) | 10/12 (83 %) | 10/12 (83 %) | 7/10 (70 %) | 10/12 (83 %) |
| CES/DES | | | | | 0 % ~ 5 % |
| PES | | | | | 0 % ~ 25 % |
| INI1+ ES | | | | | 0% |

- ES-like HE 是一种INI1+ ES 个别相似的一种肿瘤

| | AE1 / AE3 | CK | CD31 | ERG | SERPINE1-FOSB融合基因 |
|---|---|---|---|---|---|
| ES-like HE | + | + | 50% | 10/12 (83 %) | FOSB+ |
| CES/DES | + | + | - | 13/24 (54 %) | - |
| PES | + | + | - | 5/20 (25 %) | - |
| INI1+ ES | + | + | - | 0% | - |

# Conclusion

- 几乎所有上皮样肉瘤病例都显示 INI1蛋白缺失。
- 6个INI1保留的上皮样肉瘤病例中有4个发现1个或多个SWI / SNF复合物的核心亚基蛋白丢失。
- 因此， SWI / SNF复合物核心亚基蛋白的丢失可能在肿瘤发生和横纹肌样细胞形态中具有重要作用。
- 其余2 例 INI1保留的上皮样肉瘤，可能有其他异常导致SWI / SNF复合物的功能障碍。

# Thanks For Your Time