Recent advances in the diagnosis of soft tissue tumours

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Overview

- Recent diagnostic or conceptual advances in categories of
  - Peripheral nerve sheath tumours
  - Epithelioid vascular tumours
  - Adipocytic tumours
  - Round cell sarcomas
  - Myogenic sarcomas
  - Gastrointestinal stromal tumours

Malignant peripheral nerve sheath tumours

- Setting
  - Sporadic
  - In association with NF 1
  - Post radiation
- Despite diagnostic criteria dx is challenging
  - Origin from peripheral nerve or neurofibroma
  - IHC / ultrastructural evidence of Schwann cell differentiation
  - Background of NF1

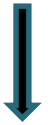
- Expression of neural markers is also limited (only 50% cases), need more specific dx tools
  - S-100
  - SOX-10
  - GFAP
- Current research aim to clarifying order of molecular events
  - CDKN2A inactivation (leading to p16 loss of function)
  - Overlapping methylation profiles

Biological progression in MPNST

Conventional neurofibroma

Atypical neurofibroma (CDKN2A)

Low grade MPNST



Intermediate MPNST

High grade MPNST

H3K27me3 loss in MPNST

Recurrent inactivating mutation of polycomb repressive complex (PRC 2) SRZ12 OR EED

↓

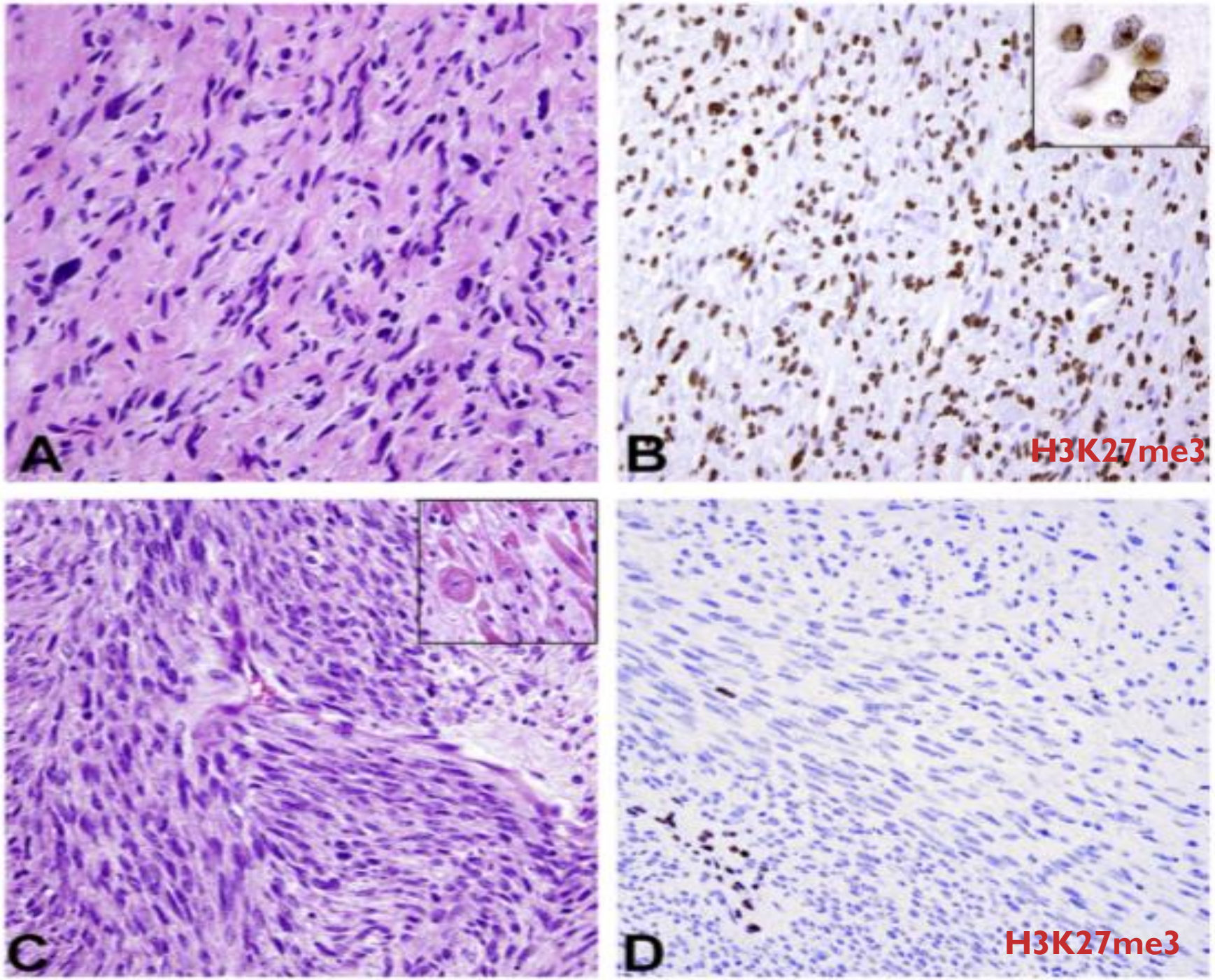
PRC2 loss of function

↓

Loss of the chromatin mark H3K27me3 (i.e trimethylation of histone 3 at lysine 27)

↓

RAS pathway activation with cooperation with CDKN2A and NF 1 inactivation

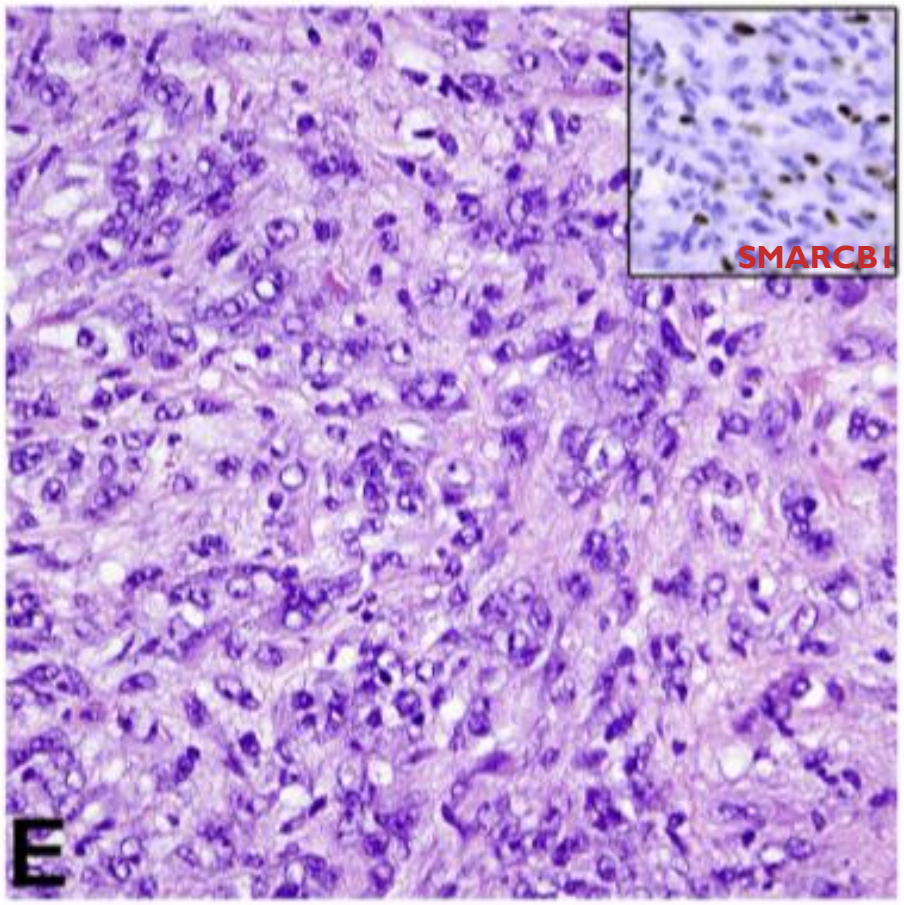

A
B
H3K27me3
C
D
H3K27me3

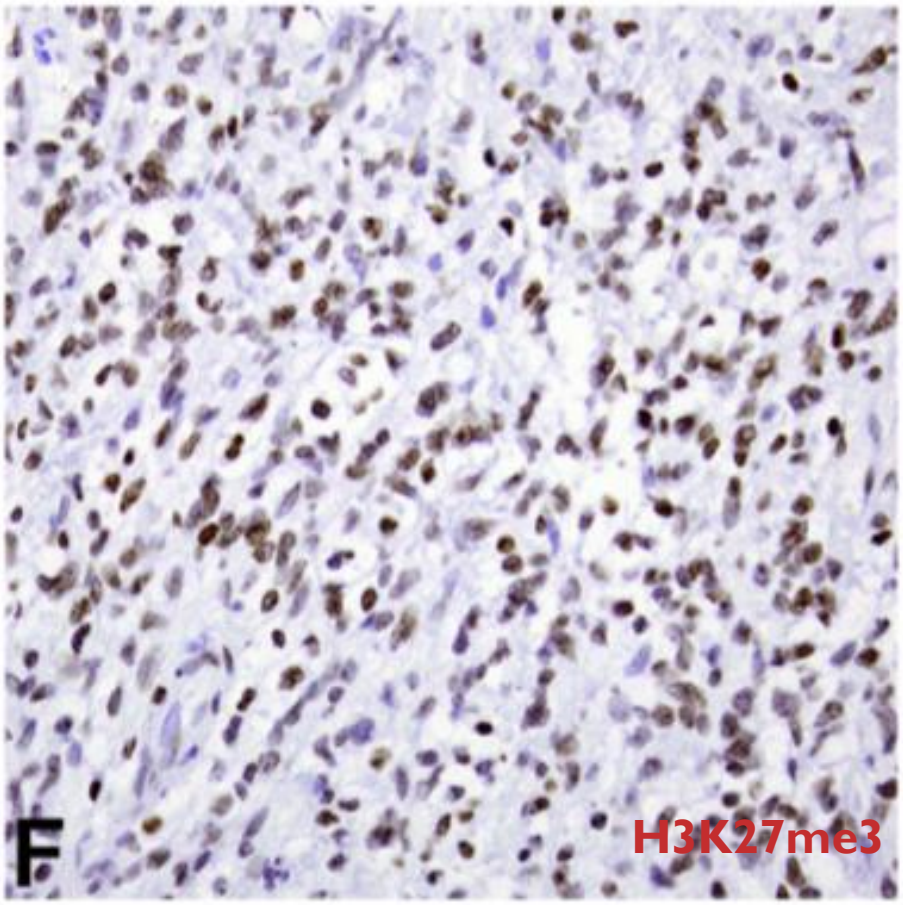
- H3K27me3 loss is very specific for MPNST(including radiation induced MPNST)
  - 30% in low grade
  - 60% in intermediate grade
  - 80% in high grade
- Other spindle cell neoplasm retain H3K27me3 including
  - Benign peripheral nerve sheath tumour
  - epithelioid MPNST
- PRC2 inactivation is not an initiating event
- Likely occur during progression from low grade to high grade.

- Other tumours showing H3K27me3 loss
  - Prepubertal pediatric nodular melanoma arising in congenital melanocytic nevus
  - Adult melanomas retained expression
  - PRC2 inactivating mutations have not been reported in melanomas
  - Epigenetic mechanisms may lead to loss in these cases
- H3K27me3 highlights
  - the inactivated X chromosome in female non-neoplastic cells
  - aid in clarification of sample identity in routine pathology setting.

SMARCB1 loss in epithelioid schwannoma and epithelioid MPNST

- Epithelioid MPNST distinct from MPNST with spindle cell morphology
  - Not associated with NF1
  - Epithelioid morphology
  - Lobulated growth pattern
  - Strong and diffuse expression of S100
  - 70% lack SMARCB 1 expression
- Epithelioid schwannoma which may arise in schwannomatosis show loss of SMARCB1 in 40% cases.


SMARCB1
E


F
H3K27me3

- SMARCB 1
  - Component of SWI/SNF1 chromatin remodeling complex
  - Master regulator of chromatin organization and accessibility
  - Function that oppose PRC2
  - Highlighting a role of epigenetic modulators in biology of PNSTs.

Molecular classification of epithelioid vascular tumours

- Epithelioid hemangioendothelioma
  - regarded as malignant
- Pseudomyogenic hemangioendothelioma
  - Multicentric
  - Rarely metastasize

Epithelioid hemangioendothelioma

- Low grade malignant endothelial neoplasm
- Predilection to soft tissue of extremities and trunk in association with large vein, also occur in lung, liver, and bone –multifocal
- Histologically
  - cords and strands of epithelioid endothelial cells with palely eosinophilic cytoplasm and intracytoplasmic vacuoles are characteristic often embedded in myxohyaline or collagenous stroma
- Express- CD 31and ERG and keratins (25%)

Molecular Profile

90% cases

↓

recurrent t(1;3)(p36.3;q25)

↓

WWTR1-CAMTA1 gene fusion

↓

CAMTA1 overexpression

5% cases

↓

t(X;11)(p11;q22) fusion event

↓

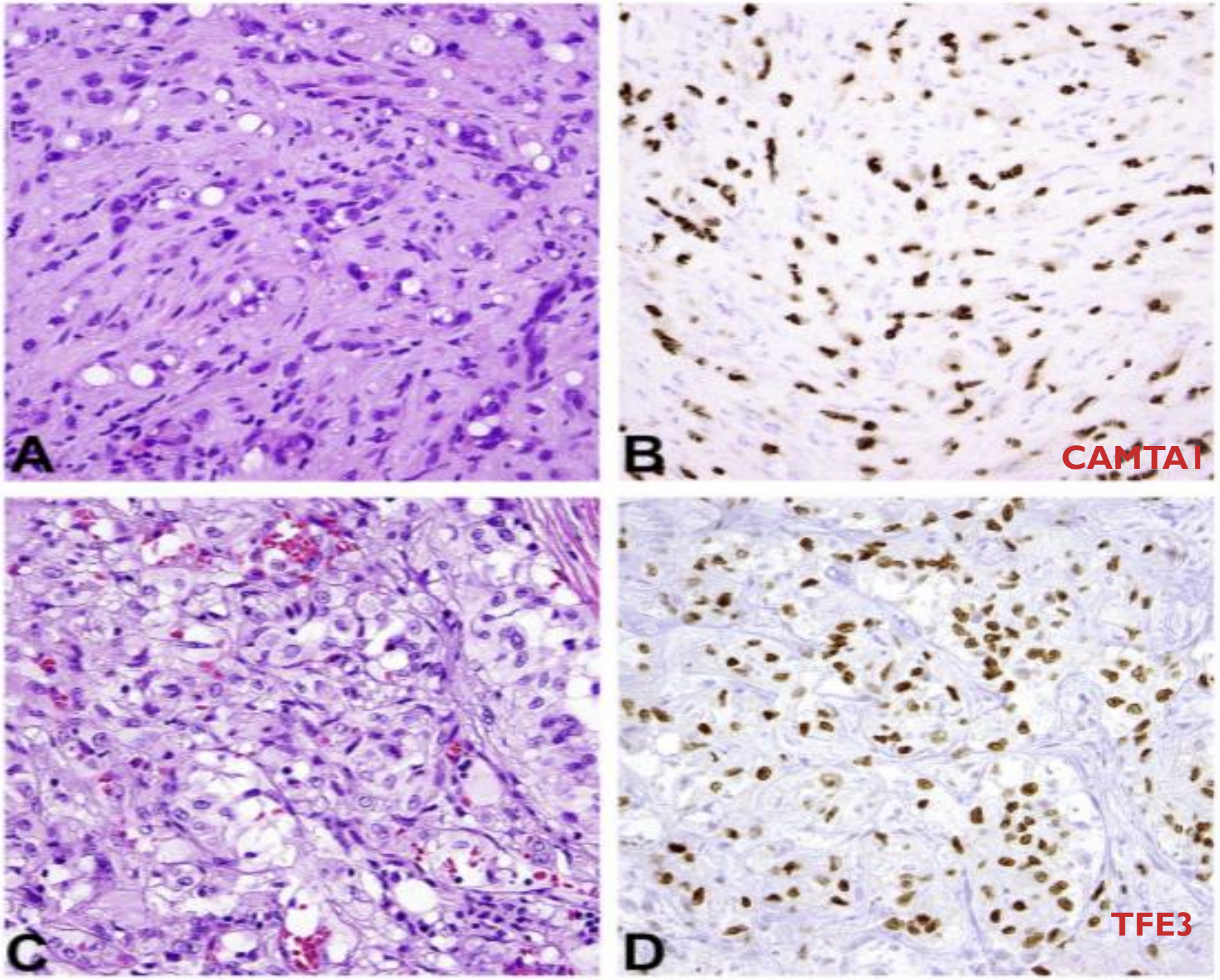
YAP1-TFE3 fusion

↓

TFE 3 overexpression (negative for CAMTA1)

- CAMTA1 IHC has recently been validated as a sensitive marker for epithelioid hemangioendothelioma

- YAP1-TFE3 fusion associated with distinct features
  - Focally well formed vascular channels
  - Tumour cells with prominent, voluminous eosinophilic cytoplasm


A
B
CAMTA1
C
D
TFE3

Epithelioid hemangioma

- Benign vascular tumour
- Commonly occur in head and neck region, trunk, limbs and deep soft tissue
- Well circumscribed, lobular mass often associated with vessel
- Histologically
  - epithelioid endothelial cells with hobnailing are found
  - Characteristic zonation of well formed vessels at the periphery and more compressed vessels in the center of the lesion
  - Nuclear atypia, pleomorphism and mitosis are mild

Variant

- Cellular variant
  - Predilection for bone and penis
  - Multifocal in 25%
  - Less vasoformative
  - Showing predominantly cellular or sheet like growth pattern

Molecular Profile

Recurrent FOSB gene rearrangements has been identified

t(19;19)(q13.2;q13.2)
OR
interstitial
del19(q13.2-3)
↓
ZFE36-FOSB gene rearrangements

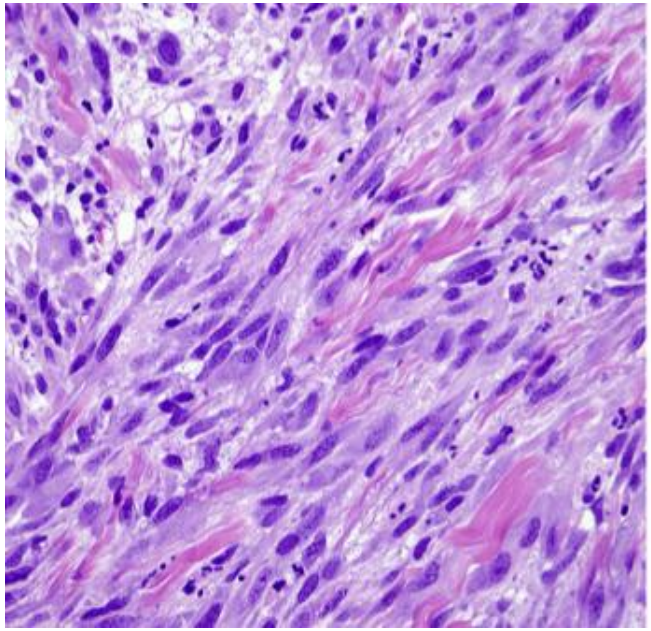
t(3;19)(q25;q12)
↓
WWTR1-FOSB gene fusion

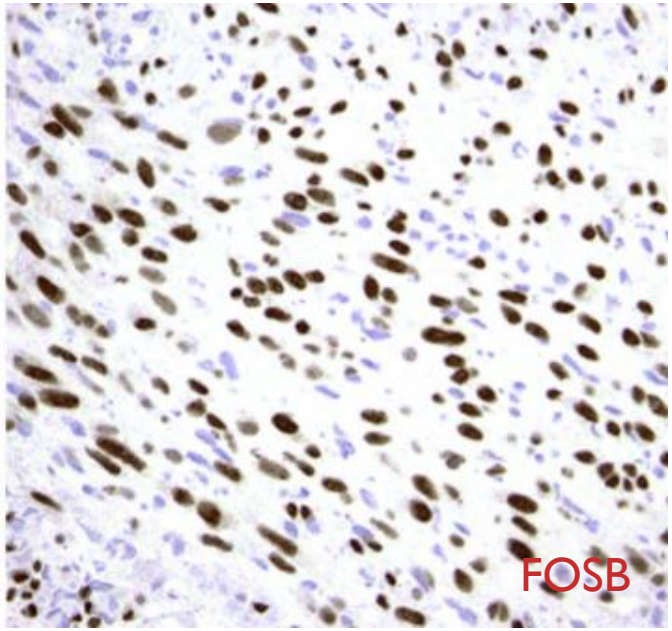
- FOSB gene rearrangements are not specific for epithelioid hemangiomas
  - Also found in pseudomyogenic hemangioendotheliomas
  - 20% cases of epithelioid hemangioma of bone and soft tissue (both cellular and conventional variant) show FOS rearrangements resulting from t(1;14)(q22;q24), t(10;14)(p13;q24) or t(3;14)(q25;24)

Pseudomyogenic (epithelioid sarcoma –like) hemangioendotheliomas

- Intermediate malignant potential
- Low distant metastatic potential
- Multiple tumours simultaneously involving various tissue planes (skin, subcutis, fascia, muscle and bone) in a given anatomical location.
- Histologically
  - loose infiltrative fascicles or sheet of plump spindled and epithelioid cells with prominent eosinophilic cytoplasm and frequent rhabdomyoblast like morphology
  - often accompanied by neutrophilc infiltrate

- Coexpression of endothelial markers such as CD31 and ERG and keratins
- Recurrent t(7;19)(q22;q13) SERPINE-FOSB1 fusion
- FOSB is expressed in all, makes highly sensitive (not specific) and diagnostically useful marker





Emerging variants of adipocytic tumours

- Spindle cell/pleomorphic lipoma
- Dedifferentiated liposarcoma
- Atypical spindle cell lipomatous tumour

Spindle cell/pleomorphic lipoma

- Predilection for the shoulder, upper back and neck region in middle aged men
- Histologically
  - composed of admixed bland spindle cells with variable amount of mature adipocytes, and additional bizzare, hyperchromatic to multinucleate cells in pleomorphic lipoma
  - Tumour cells show short stubby nuclei and indistinct cytoplasm surrounded by fibromyxoid stroma with prominent ropey collagen bundles
  - lipoblast may be present
- Loss of RB 1 caused by 13q14 deletion, translates into RB loss by IHC

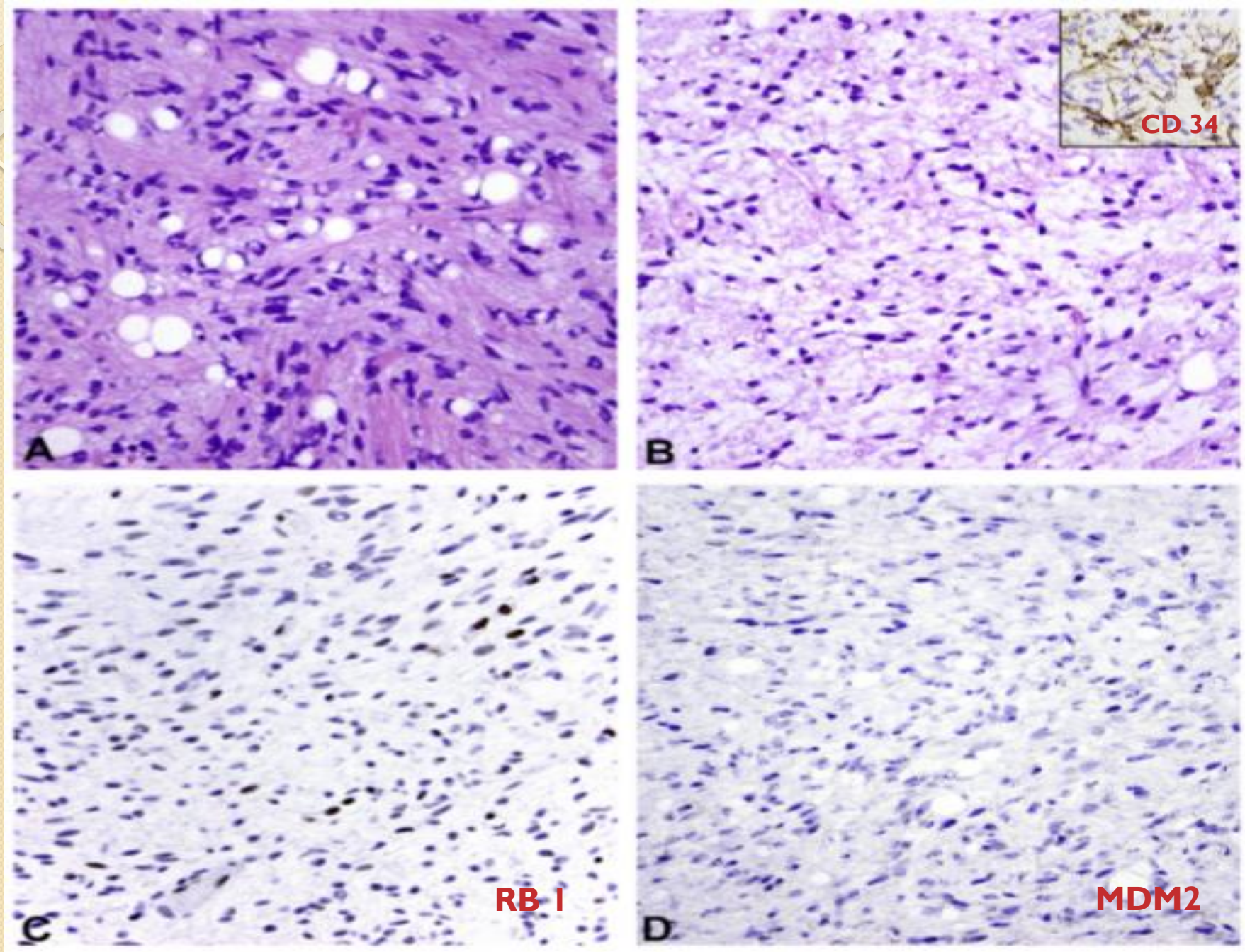
Dedifferentiated liposarcoma

- Affects middle aged to older adults
- Deep soft tissues of retroperitoneum, spermatic cord, extremities, mediastinum and head and neck region
- arises in well differentiated liposarcoma/ atypical lipomatous tumour
- Cytogenic alteration
  - giant or ring chromosome that contain amplified material from 12q13-15 which includes the MDM2, CDK4 and HMGA2 loci.
  - These abnormalities result in overexpression of MDM2 and CDK4 (and HMGA2) detectable by IHC

Atypical spindle cell lipomatous tumour

- Does not fit in any existing category
- Risk of local recurrence but lack of dedifferentiation or distant metastasis
- Extremities, limb girdle, hand and feets
- Histologically poorly marginated
  - consist of atypical spindle cells embedded in a fibrous or myxoid stroma
  - variably prominent adipocytic component showing variation in adipocyte size and focal nuclear atypia frequently with univacoulated or mutivacuolated lipoblasts

- Expression of CD34 and loss of RB1
- Lack coexpression of MDM2 and CDK4.
- 10% show focal expression of either MDM2 or CDK4 by IHC but FISH is negative for high level of MDM2 amplification.
- Staining for S100 (40%) and desmin (20%) may be observed.


A
B
CD 34
C
RB 1
D
MDM2

- Indolent behavior
- Highlights the importance of distinguishing atypical spindle cell lipomatous tumour from atypical lipomatous tumour
- To avoid aggressive surgical resections
- Excellent prognosis if resection is complete

Expanding spectrum of round cell sarcomas

- Round cell sarcomas characterized by sheets of poorly differentiated cells with small, blue, round nuclei and scant cytoplasm includes
  - Classic Ewing's sarcoma
  - Round cell sarcoma with CIC rearrangement
  - Round cell sarcoma with BCOR rearrangement

Classic Ewing's sarcoma

- Osteomyelitis of femur (14 yrs) spent several years at bed rest being tutored and entering contests, he won a microscope in one contest, later choice of carrier as a pathologist
- "for some years I have been encountering material curetted from bone tumours a structure which differed markedly from that of osteogenic sarcoma, was not identical with any form of myeloma ,and which had to be designated by the vague term 'round cell sarcoma' of unknown origin and nature."


James Ewing (1866-1943)

Classic Ewing's sarcoma

- Uniform cells with rounded nuclei and inconspicous nucleoli in diffuse sheets with variable necrosis
- Rearrangements involving ESWR1 in majority (90%) with ESWR1-FLI 1 fusion resulting from t(11;22)(q24;q12)
- Strong diffuse, membranous expression of CD99 (not specific)
- nuclear expression of transcription factor NKX2-2

Round cell sarcomas with CIC rearrangements

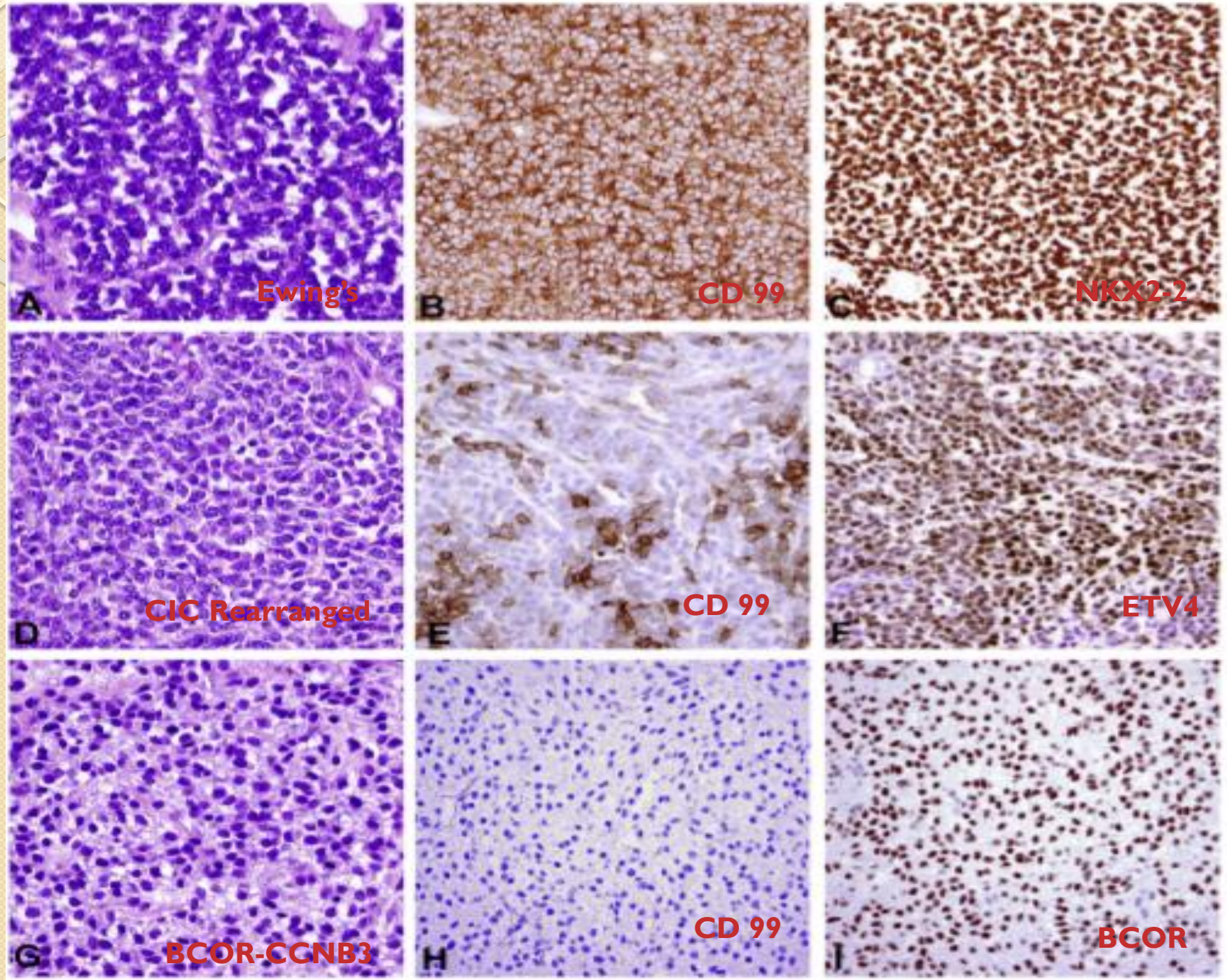
- Predilection for the soft tissue of trunk and extremities of younger male adults
- Molecular profile
  - Lacking ESWR1 rearrangements
  - Recurrent CIC rearrangements with t(4;19)(q35;q13) or t(10;19)(q26;q13) resulting most commonly in CIC-DUX4 fusion
- Histologically
  - primitive ovoid or sometimes spindled morphology, irregular shaped vesicular nuclei with coarse chromatin and prominent nucleoli, areas of necrosis and frequent mitosis.

- Characteristic features
  - High degree of morphological heterogeneity,
  - distinct nucleoli, more abundant cytoplasm
  - Strong and diffuse nuclear staining for WT1, ETV4 and CIC
  - limited CD99 expression are characteristic
  - NKX2-2 expression in only a small subset
- Behavior
  - aggressively with lower overall survival
  - managed clinically in similar fashion like Ewing's
- Novel treatment approaches will need to develop as these tumors quite often develop resistance to t/t protocol for Ewing's sarcoma

Round cell sarcoma with BCOR rearrangement

- Predilection for bone and soft tissue of male children
- Molecular profile
  - Lacking ESWR1 and CIC rearrangements
  - BCOR-CCNB3 fusion resulting from inv (X)(p11) (i.e- X chromosomal paracentric inversion)
  - Rarely alternate MAML3 or ZC3H7B genes.
- Histologically
  - monomorphic or primitive appearing round to ovoid and occasionally spindled tumour cells arranged in intersecting fascicles or a patternless fashion.

- BCOR IHC detect protein overexpression in all cases
- Variable expression of CD99
- lack NKX2-2, ETV4 and WT1
- Other tumour with ZC3H7B-BCOR fusion
  - Ossifying fibromyxoid tumour
  - High grade endometrial stromal sarcomas
- Behave aggressively with 5 year survival rate 77%


A
Ewing's
B
CD 99
C
NKX2-2
D
CIC Rearranged
E
CD 99
F
ETV4
G
BCOR-CCNB3
H
CD 99
I
BCOR

MYOD1 mutations in spindle cell/ sclerosing rhabdomyosarcomas

- Previous molecular spectrum of rhabdomyosarcomas
  - Embryonal rhabdomyosarcoma with frequent activating the RAS signaling pathway
  - Alveolar rhabdomyosarcoma PAX3-FOXO1 fusions or PAX7-FOXO1 fusions
  - Pleomorphic rhabomyosacoma
  - Spindle cell rhabdomyosarcomas (previously variant of embryonal RMS)

International classification of rhabdomyosarcoma

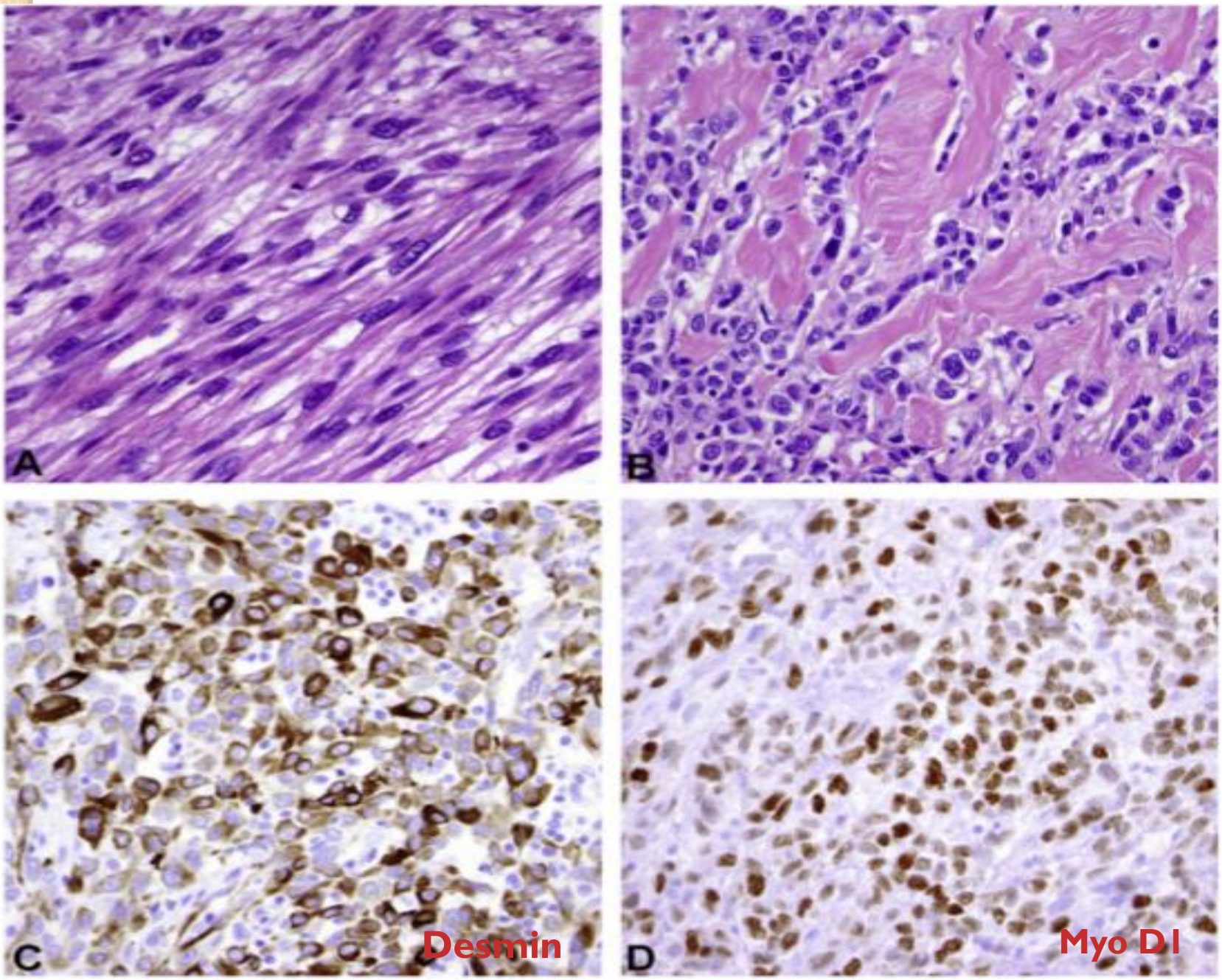
- Superior prognosis
  - Botryoid RMS
  - Spindle cell RMS
- Intermediate prognosis
  - Embryonal RMS
- Poor prognosis
  - Alveolar RMS
  - Undifferentiated Sarcoma
- Subtypes whose prognosis is not presently evaluable
  - RMS with rhabdoid features

Spindle cell rhabdomyosarcomas

- Distinct subtype
- Children
  - Paratesticular region
  - Indolent course
- Adults
  - head and neck region
  - more aggressive course
- Histologically two cell populations comprising
  - dominant spindle cell population forming long, intersecting fascicles with ovoid to elongated nuclei and pale cytoplasm
  - Minor population of rhabdomyoblasts with hyperchromatic eccentric placed nuclei and abundant eosinophilic cytoplasm

- **Sclerosing RMS** is a morphological variant
  - Affecting adults and children
  - Ovoid to rounded tumour cells with small amount of cytoplasm often arranged in nests and embedded in a densly hyalinised stroma
- Both sclerosing and spindle cell appearances have been observed in the same tumour
- Now considered two points along a histological spectrum
- Classified separately as a single entity in WHO.

- IHC
  - Desmin
  - myogenic transcripton factor myf-4(myogenin)
  - MyoD1(strong and diffuse)
- Molecular profile
  - Small subset in neonates and infants recurrent NCOA2 gene rearrangements
  - Recurrent MYOD1 genomic alterations


A
B
C
Desmin
D
Myo D1

Activating p.L122R mutation in MYOD1

↓

result in activation of MYC- like transcriptional program

↓

switch from differentiation to proliferation

↓

suggested by spindle cell morphology and limited degree of sarcometric differentiation

- MYOD1 mutations were found in association
  - PIK3CA
  - PTEN Deletions
  - Altering PI3K-AKT pathway signaling
- Common molecular basis
  - Both variants represents single pathological entity
  - Help to separate from other variants

KIT/PDGFRA wild type GIST

- Identification of interstitial cells of cajal (ICC)
  - with which GIST share expression of CD 34 and KIT
  - made them different from other soft tissue tumour
- 'Micro GIST' (< 1.0 cm)
  - 30% common in general population
- <0.1% progress to clinically relevant tumor
- 85% Oncogenic KIT and PDGFRA driver mutations
  - successful introduction of imatinib as first line therapy
- Two major subgroup
  - NF1 associated GIST
  - SDH deficient GIST

Genomic progression

Initial event -Oncogenic tyrosine kinase mutations(present in micro GIST)

↓

Chromosomal losses at 14q,22q,1p and 15q harbor putative tumour suppressor genes

↓

Inactivation of MAX(on 14q) and dystrophin (encoded by DMD on Xp) tumour suppressor functions

↓

Early and late events in GIST progression

NF 1 associated GIST

- Associated NF 1 tumor syndrome
  - Predilection for small intestine
  - Spindle cell morphology
  - Multifocality
- Low risk rarely metastasize.
- Biallelic NF1 inactivation which supplants the lack of constitutive tyrosine kinase by downstream activation of same signaling pathways

- Sporadiac GIST lacking mutations in KIT, PDGFRA, SDH or BRAF
- Have germline mutations NF1 mutations
- Despite lack of driver mutation KIT show strong expression of KIT(and also DOG1) by IHC
- Generally resistant to TKI therapies

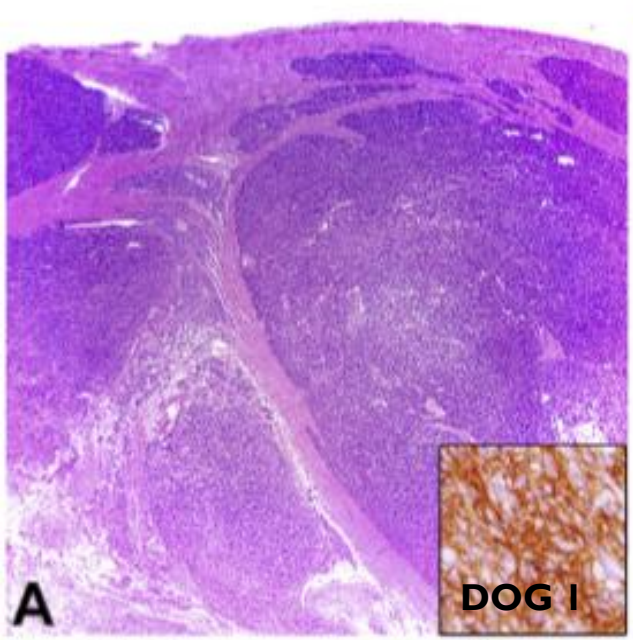
SDH deficient GIST

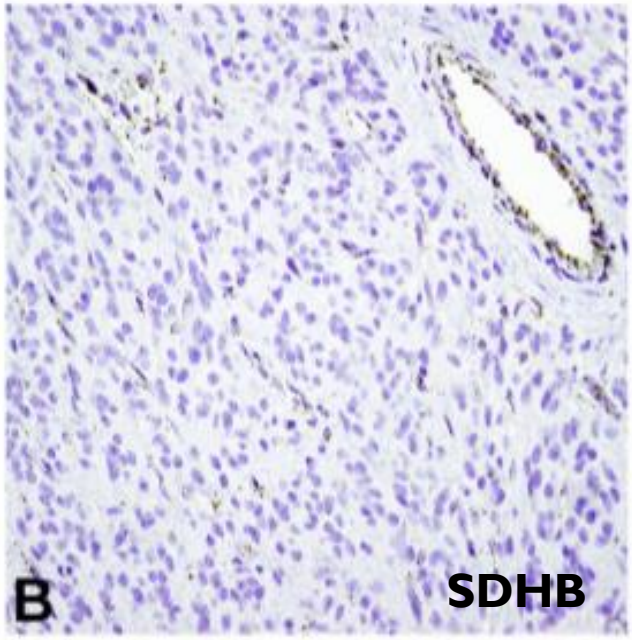
- Loss of function alterations of SDH, an enzymatic complex involved in citric acid cycle and electron transport chain.
- Loss of function of any four SDH complex subunits(encoded by SDHA, SDHB, SDHC,SDHD)
  - results in loss of SDHB expression
- 80% cases inactivating SDH subunit mutations
- 20% SDHC promotor methyaltion lead to epigenetic SDHC inactivation.

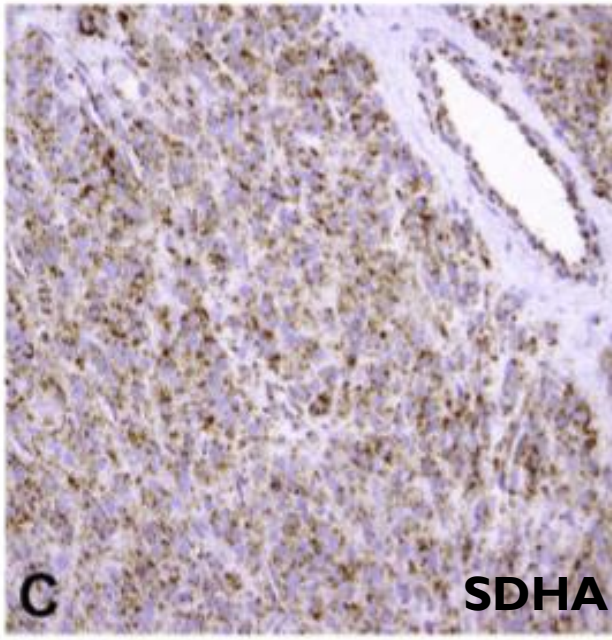
- Loss of SDHB confirms SDH deficiency but non specific
- Additional loss of SDHA expression indicates an underlying SDHA mutation
- Where as SDHB,SDHC,SDHD mutated/ epimutated GIST retain SDHA expression
- Lack characteristic chromosomal alterations of conventional GIST and show loss at 1p or 1q(include SDHC locus)

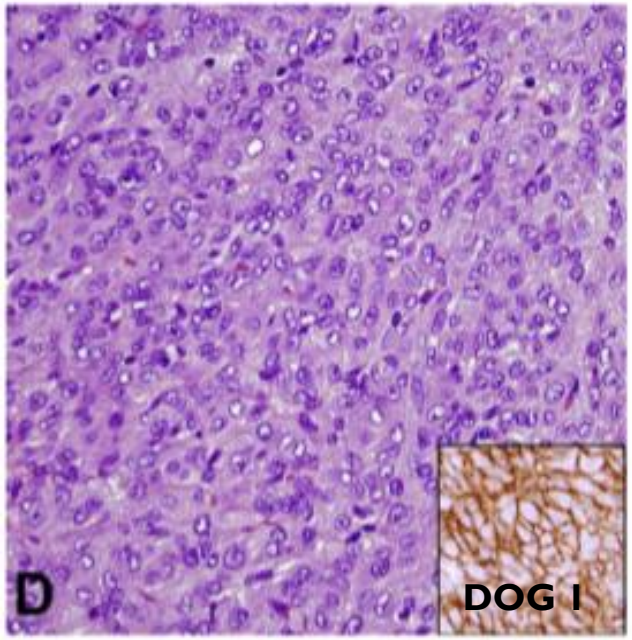
- SDH deficient GIST occur in association with
  - Nonhereditary carney triad together with paraganglioma and pulmonary chondroma
  - AD Carney- Stratakis syndrome (in association with paraganglioma) who harbor SDH subunit germline mutations
- In pediatric patients
  - Majority are wild type for KIT and PDGFRA
  - Most are SDH deficient
  - In young girls
  - carney or carney stratakis syndrome

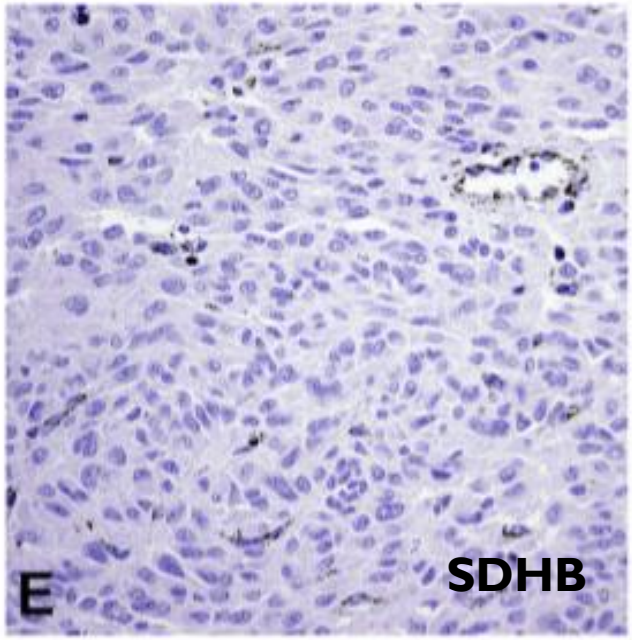
- Different clinical and histomorphological features
  - Always arise in stomach
  - Epithelioid or mixed morphology
  - Typical multinodular or plexiform growth pattern with in muscularis propria which facilitates their recognition at low power
  - Show expression of KIT and DOG 1
  - Resistant to TKI therapies
  - Despite propensity to lymph node metastasis and multifocal presentation they follow indolent course. (risk stratification does not applied)


A
DOG I


B
SDHB


C
SDHA


D
DOG I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SDHB

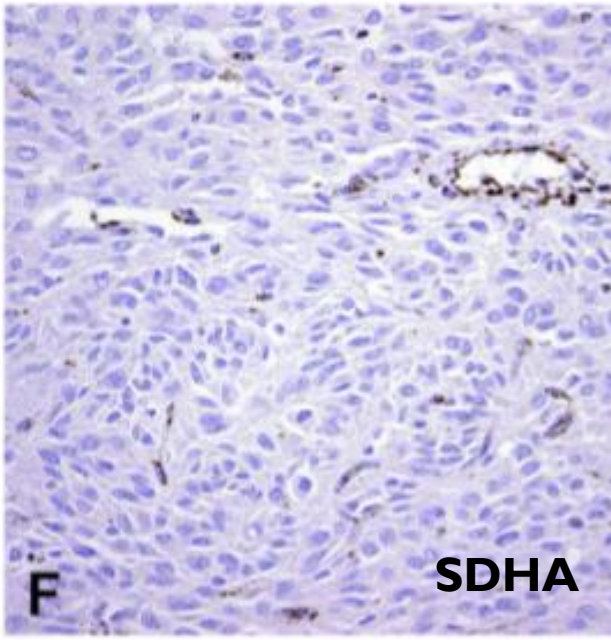
F
SDHA

Table 1 Overview of recently characterised entities and related biomarkers

Tumour type	IHC	Staining pattern	% of cases	Other useful markers	Genetics
Neural tumours					
MPNST	H3K27me3	Loss	30% low grade, 60% intermediate grade, 80% high grade	S-100, SOX10, GFAP (all subset only, <50% of cases)	*SUZ12* or *EED* mutation (PRC2 inactivation); *NF1* inactivation
Epithelioid MPNST	SMARCB1	Loss	70%	S-100 (strong, diffuse)	
Vascular tumours					
Epithelioid haemangioendothelioma	CAMTA1; TFE3	Overexpression	90%; 5%	CD31, ERG	*WWTR1-CAMTA1* fusion; *YAP1-TFE3* fusion
Epithelioid haemangioma	FOSB	Overexpression	50%	CD31, ERG	*ZFP36-FOSB* fusion; *WWTR1-FOSB* fusion; *FOS* rearrangement
Pseudomyogenic haemangioendothelioma	FOSB	Overexpression	96%	CD31, ERG, keratin	*SERPINE1-FOSB* fusion
Adipocytic tumours					
Atypical spindle cell lipomatous tumour	RB1	Loss	60%	CD34, desmin, S-100 (subset)	13q14 deletion
Round cell sarcomas					
Ewing's sarcoma	NKX2-2	Nuclear overexpression	>90%	CD99 (diffuse, membranous)	*EWSR1-FLI (90%); ESWR1-ERG (5%);* others
Sarcoma with *CIC* rearrangement	WT1, ETV4	Nuclear overexpression	>90%	CD99 (limited)	*CIC-DUX4* fusion (rarely *CIC-FOXO4* fusion)
Sarcoma with *BCOR* rearrangement	BCOR	Nuclear overexpression	>90%	CD99 (variable)	*BCOR-CCNB3* fusion (*BCOR-MAML3*; *ZC3H7B-BCOR*)
Myogenic sarcomas					
Spindle cell/sclerosing rhabdomyosarcoma	MYOD1	Overexpression	100%	Desmin, myf-4	*MYOD1* mutation (p.L122R)
GIST					
SDH-deficient GIST	SDHB (SDHA)	Loss	~90% of *KIT/PDGFRA* wild-type GIST	KIT, DOG1	*SDHA/SDHB/SDHC/SDHD* mutation/*SDHC* hypermethylation

GIST, gastrointestinal stromal tumour; IHC, immunohistochemistry; MPNST, malignant peripheral nerve sheath tumour; NF1, neurofibromatosis type 1; PRC2, polycomb repressive complex 2; SDH, succinate dehydrogenase complex.

THANK YOU