Introduction to Soft Tissue Pathology

By Konstantinos Linos M.D.

General considerations

- Soft tissue can be defined as **nonepithelial extraskeletal tissue** of the body.

- It is represented by the voluntary **muscles**, **fat**, and **fibrous tissu**e, along with the **vessels** serving these tissues.

- By convention, it also includes the **peripheral nervous system**

General considerations

- Soft tissue tumors are a **highly heterogeneous** group of tumors.

- They are classified by the **line of differentiation**, according to the adult tissue they resemble.

Benign

- Benign tumors have **a limited capacity for autonomous growth**.
- They exhibit little tendency to **invade locally**
- They are attended by **a low rate of local recurrence following conservative therapy**.

Malignant

- Malignant tumors, or sarcomas are locally aggressive and **capable of invasive or destructive growth, recurrence, and distant metastasis**.

- **Radical surgery** is required to ensure the total removal of these tumors.

- The term **sarcoma** does not indicate the likelihood or rapidity of metastasis.

- For these reasons, it is important to qualify the term sarcoma with a statement concerning the **degree of differentiation or the histologic grade**.

- **Histologic grade** is a means of quantitating the degree of differentiation by applying a set of histologic criteria.

- Tumors of **intermediate or borderline** malignancy are characterized by frequent recurrence but rarely metastasis.

Pathogenesis

- **Enviromental factors**
- **Vinyl chloride**
- **Radiation exposure**
  - Documentation within radiation field
  - Period latency of at least three years
  - Most high grade lesions (70% undifferentiated pleomorphic sarcoma)

Pathogenesis

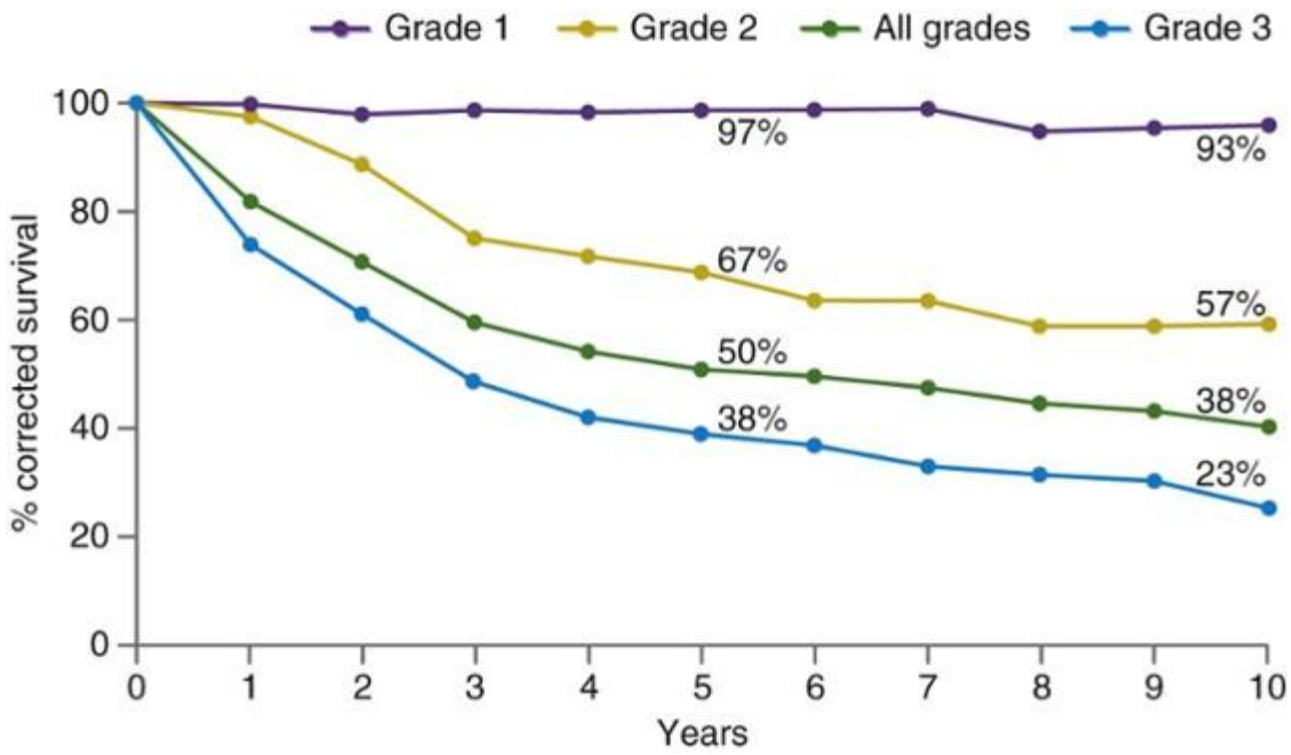
- **Oncogenic viruses**
  - Kaposi sarcoma and HHV8
  - EBV associated smooth muscle tumors
- **Immunologic factors**
- **Genetic factors**
  - Neurofibromatosis 1, Familial adenomatous polyposis (FAP)/Gardner syndrome

Classification of Soft Tissue Tumors

- Most tumors retain the same pattern of differentiation in the primary and recurrent lesions
- **Undifferentiated pleomorphic sarcoma** and **liposarcoma** are the most common soft tissue sarcomas of adults
- **Rhabdomyosarcoma**, **neuroblastoma**, and the **Ewing family of tumors** are the most frequent soft tissue sarcomas of childhood.

Grading and Staging

- **Grading**
  - Assessment of the degree of malignancy of a sarcoma
- **Staging**
  - Provides shorthand information regarding the extent of the disease at a designated time


Grade 1
Grade 2
All grades
Grade 3
% corrected survival
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
Years
97%
93%
67%
57%
50%
38%
38%
23%

Grading and Staging

Grading System

- **Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC)**

Staging System

- **TNM classification of tumors 7th edition (TNM)**

Histologic Features evaluating in Grading (FNLCC)

- Differentiation
  - **Score 1**: Sarcoma histologically very similar to normal adult mesenchymal tissue
  - **Score 2**: Sarcoma of defined histological subtype
  - **Score 3**: Sarcoma of uncertain type, embryonal and undifferentiated sarcomas

Histologic Features evaluating in Grading (FNLCC)

- **Mitotic Index per 10 High-Power Fields** (HPF)

  -- Score 1: 1-9 mitoses

  -- Score 2: 10-19 mitoses

  -- Score 3: 20 or more mitoses

  Total number in 10 successive HPF in most mitotically active areas

  Avoid ulcerated, necrotic, or hypocellular areas

Histologic Features evaluating in Grading (FNLCC)

- **Percentage of Microscopic Tumor Necrosis**
  - Score 0: No necrosis
  - Score 1: Necrosis < 50%
  - Score 2: Necrosis > 50%

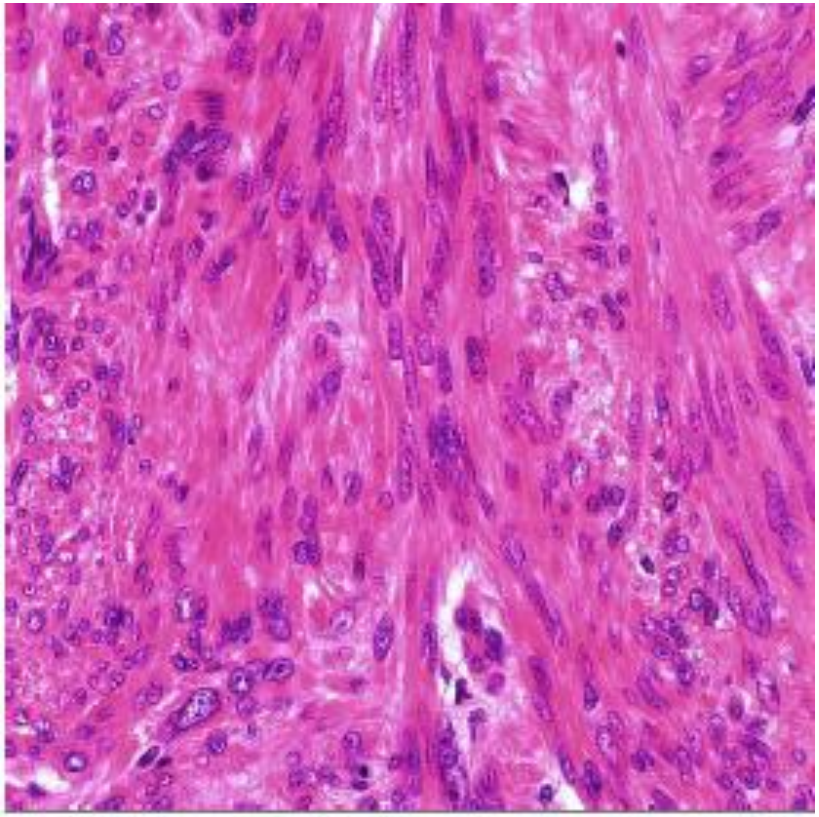
Histologic Features evaluating in Grading (FNLCC)

- Summation of 3 scores indicates grade
  - **Grade 1**: Total score 2 or 3
  - **Grade 2**: Total score 4 or 5
  - **Grade 3**: Total score 6, 7, or 8

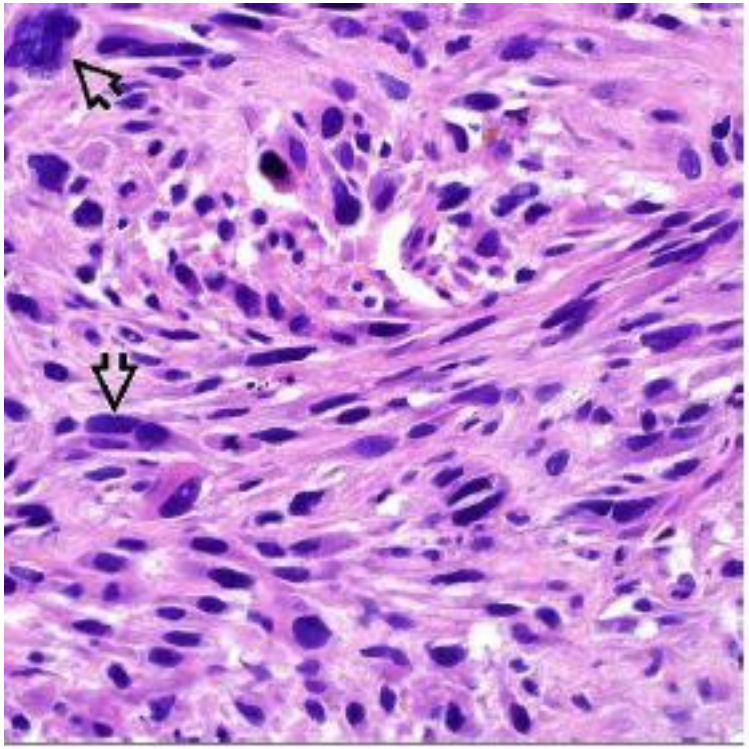
  – In practice, some tumors are always graded by diagnosis

- This is leiomyosarcoma with a differentiation score of 1.

- The tumor is composed of well-organized fascicles of spindle cells with uniform nuclei and abundant cytoplasm, and it resembles normal smooth muscle



- This is leiomyosarcoma with a differentiation score of 3.
- A fascicular architecture is only focally present.
- The cells show high nuclear/cytoplasmic ratio and focally marked nuclear pleomorphism .



FNCLCC system

- Its principal weakness lies in the assignment of the differentiation score.

- This system has been derived from resected specimens **unmodified by treatment**

Limitations of Grading

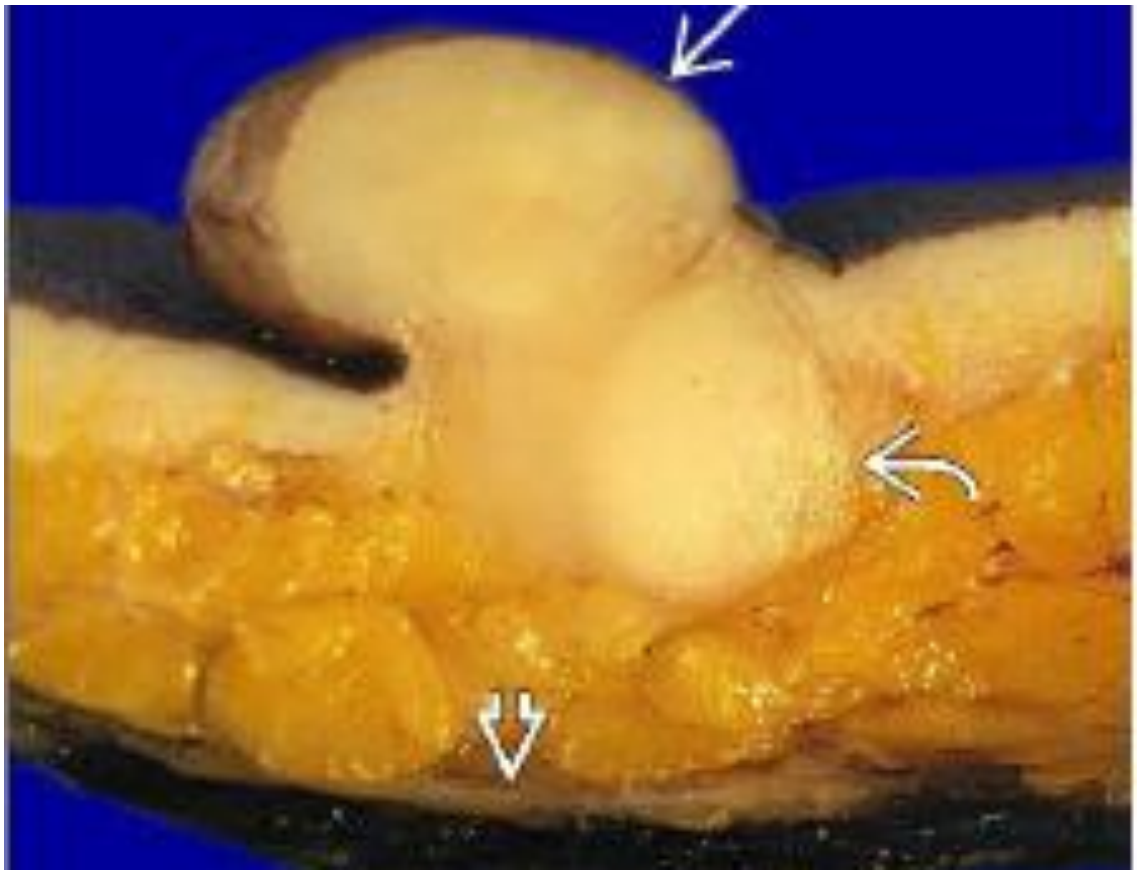
- No grading system performs well on every type of sarcoma.
- Some rare sarcomas are considered difficult, if not impossible, to grade.
- In a number of sarcomas, clinical features play a larger role in determining a prognosis

Limitations of Grading

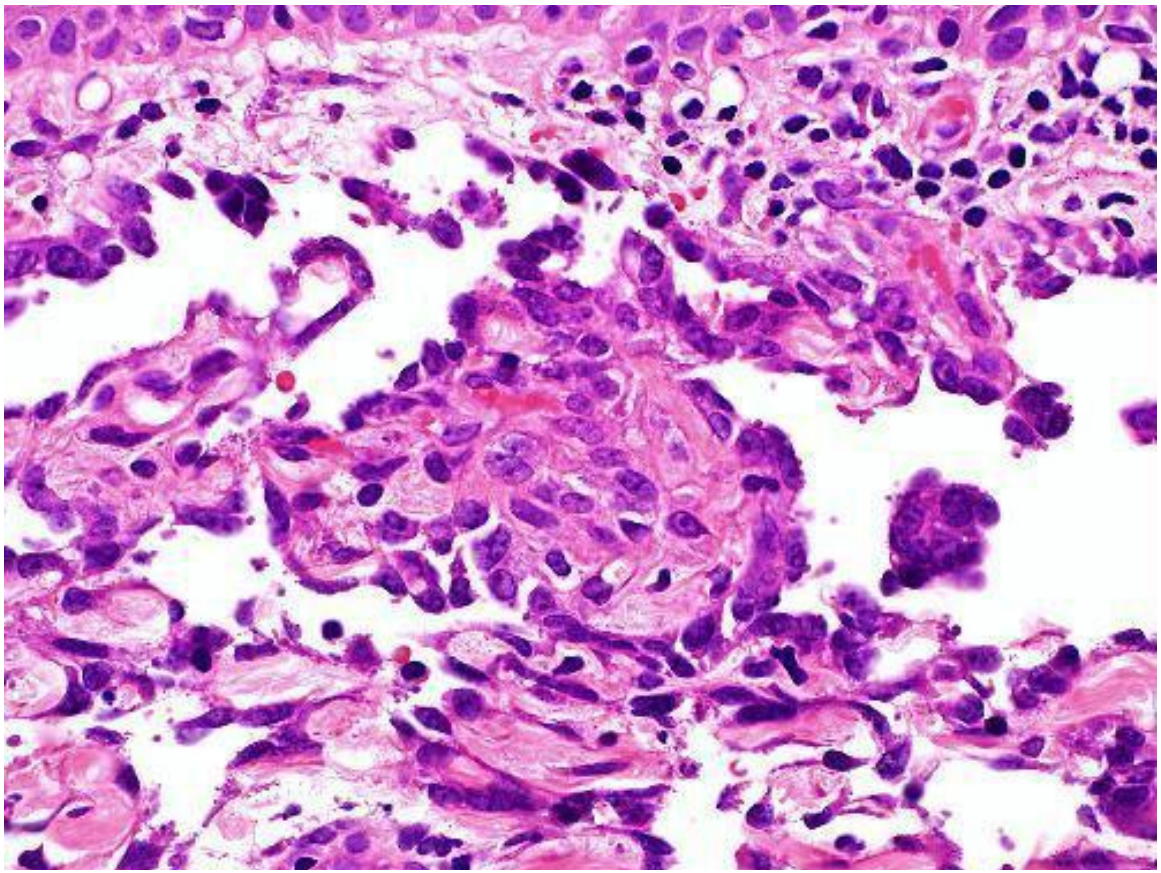
- Necrosis &mitoses
  - Selection of the tissue sample and the length of fixation
  - Necrosis may be prominent in tumors of which a biopsy has been previously performed or that have been **irradiated or embolized**
- Grading is usually based on **the least differentiated area** of a tumor, unless it comprises a very minor component of the overall tumor

Limitations of Grading

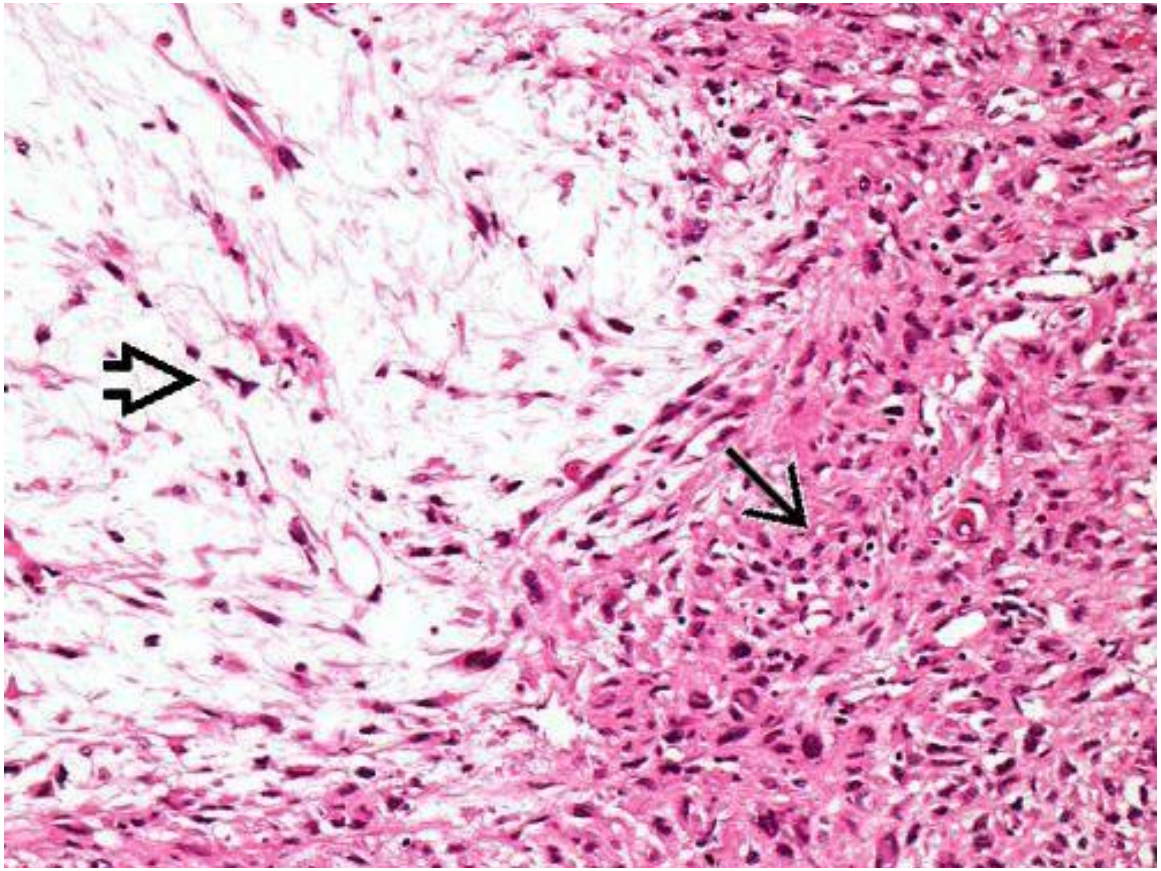
- Grading remains one of the most powerful and inexpensive ways of assessing the prognosis in a sarcoma
- It is currently regarded as **a major independent predictor of metastasis** in the major histologic types of adult soft tissue sarcomas
- Grade should be provided by the pathologist, whenever possible



This example of dermatofibrosarcoma protuberans involves the skin and subcutis but does not extend to deep fascia



This cutaneous angiosarcoma is well differentiated but should be managed as a high-grade malignancy.



This shows myxofibrosarcoma with low- and high-grade areas. The latter display increased cellularity, pleomorphism, and mitoses

Cytogenetic and Molecular Genetic Pathology of Soft tissue tumors

- Self-sufficiency in growth signals
- Insensitivity to growth-inhibitory signals
- Evasion of programmed cell death (apoptosis)
- Limitless replicative potential
- Sustained angiogenesis
- Tissue invasion and metastasis

General concepts in cancer genetics

- Multistep carcinogenesis which encompasses a progression from preneoplastic lesions to invasive cancer **does not** translate well to sarcomas

Translocation associated sarcomas

- **In translocation sarcomas no preneoplastic phase is recognized**

- In many cases the translocation is the only identifiable genetic alteration

- This suggests that the translocation may, by itself, provide several hallmarks of cancer

Genetic alterations and cancer

- **Oncogenes**
- **Tumor suppressor genes**
- **Caretakers genes**

Oncogenes

- **Mutation**
- Increase number of copy gene (**amplification**)
- **Translocation** (brings its expression under the control of the regulatory sequences (promoter) of another gene
- **Combination** of these

Examples

- **KIT** and **PDGFRA** both activated by mutations (e.g GIST)
- **MYCN** and **MDM2** both activated by gene amplifications (e.g angiosarcoma and liposarcoma)
- **PLAG1** and **HMGA1**, activated by translocation-mediated promoter juxtaposition (e.g pleomorphic adenoma and lipoma)

Tumor suppressor genes

- Mutation-> **truncated or inactive protein**
- Mutation-> **dominant negative protein** that interferes with the function of the normal protein
- **Large deletions**
- Replacement of the remaining unmutated copy by the inactive copy (**loss of heterozygosity**)
- Reduced expression caused by **hypermethylation** of the regulatory sequences of the gene
- Interruption of the gene by **non-recurrent translocation**

Key examples in sarcomas

- Generic tumor suppressors: TP53 (p53), RB1 and CDKN2A (p16)
- Sarcoma-selective suppressors : NF1 and SMARCB1 (INI1)
- **Classical tumor suppressor genes act through complete or near complete loss of function of the protein**

Caretaker genes

- Contribute to oncogenesis through **a loss of function mechanism**

- The loss of function of caretakers genes **increases the likelihood that oncogene activation or conventional tumor suppression will occur**

Functional classes

- The vast majority of cancer genes belong to one of four groups:
  - **Protein kinases** (oncogenes or tumor suppressor)
  - **Transcription factors** (oncogenes or tumor suppressor)
  - **DNA maintenance and repair proteins** (caretaker genes)
  - Cancer genes involved in **cellular metabolism** (e.g IDH1, IDH2, SDH)

	SARCOMAS WITH SPECIFIC TRANSLOCATIONS	SARCOMAS WITH OTHER GENETIC ALTERATIONS
Karyotypes	Usually simple	Usually complex
Translocations	Reciprocal and specific, producing fusion genes	Nonreciprocal and nonspecific, causing gene copy number changes
Telomere maintenance mechanisms	Telomerase expression common, ALT mechanism rare	ALT mechanism more common than telomerase
P53 pathway alterations	Relatively rare, but strong prognostic impact	More frequent, but limited or no prognostic impact
Incidence in bilateral retinoblastoma and Li-Fraumeni syndrome	Rare, if ever	Common
Similarity of gene expression profiles within each sarcoma type	Strong	Weak

ALT, alternative lengthening of telomeres.

General principles of translocations

- Biologically these gene fusions operate either by:
  - **Overexpression of the encoded protein**
  - **Promoter substitution**
  - **Formation of aberrant chimeric proteins**

General principles of translocations

- Chromosomal translocations constitute the **majority of specific genetic alterations associated with sarcomas**

- Produce highly specific gene fusions

- **The specificity of these gene fusions and their prevalence in selected sarcomas have become a defining feature of many of these entities**

Key concepts in translocation associated sarcomas

- They **contain their fusion gene from their earliest presentation and do not show a benign or premalignant phase**

- The fusion gene is **present in all tumor cells** and is expressed throughout the clinical course

TRANSLOCATIONS AND OTHER GENETIC ANOMALIES IN SARCOMAS

Diagnostic Molecular and Cytogenetic Findings in Sarcomas

Histologic Type	Translocation or Rearrangement	Fusion Gene or Other Feature
Alveolar soft part sarcoma	t(X;17)(p11;q25)	*ASPL-TFE3*
Angiomatoid fibrous histiocytoma	t(12;22)(q13;q12)	*EWSR1-ATF1*
	t(12;16)(q13;p11)	*FUS-ATF1*
	t (2;22)(q33;q12)	*EWSR1-CREB1*
Clear cell sarcoma of soft parts	t(12;22)(q13;q12)	*EWSR1-ATF1*
Clear cell sarcoma (gastrointestinal)	t(2;22)(q33;q12)	*EWSR1-CREB1*
Dermatofibrosarcoma protuberans and variants	t(17;22)(q21;q13)	*COL1A1-PDGFB*
	Ring form of chromosomes 17 and 22	
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	*EWSR1-WT1*
	t(21;22)(q22;q12)	*EWSR1-ERG*
Endometrial stroma sarcoma	t(7;17)(p15:q21)	*JAZF1-JJAZ1*
	t(6;7)(p21;p22)	*JAZF1-PHF1*
	t(10:17)(q22;p13)	
Epithelioid hemangioendothelioma	t(1;3)(p36.3;q25)	
	t(10;14)(p13;q24)	Gene for VEGF-related protein at 14q24
Epithelioid sarcoma	Abnormalities of 22q	*INI1* inactivation
Ewing sarcoma/primitive neuroectodermal tumor	t(11;22)(q24;q12)	*EWSR1-FLI1*
	t(21;22)(q12;q12)	*EWSR1-ERG*
	t(2;22)(q33;q12)	*EWSR1-FEV*
	t(7;22)(p22;q12)	*EWSR1-ETV1*
	t(17;22)(q12;q12)	*EWSR1-E1AF*
	inv(22)(q12;q12)	*EWSR1-ZSG*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	*EWSR1-NR4A3*
	t(9;17)(q22;q11)	*TAF1168-NR4A3*
	t(9;15)(q22;q21)	*TCF12-NR4A3*
Fibrosarcoma, infantile	t(12;15)(p13;q26)	*ETV6-NTRK3*
	Trisomies 8, 11, 17, and 20	
Gastrointestinal stromal tumor		*KIT, PDGFRA* mutations
Inflammatory myofibroblastic tumor	2p23 rearrangement	*ALK* fusions with various genes

Leiomyosarcoma	Deletion of 1p	
Liposarcoma		
Well-differentiated	Ring form of chromosome 12	12q13-15 amplicon including *MDM2, CDK4, HMGA2*
Dedifferentiated	Ring forms and complex changes	12q14 amplicon including *MDM2, CDK4, HMGA2, ASK1,* and *JUN* amplification
Spindle cell		*RB1* deletion
Myxoid/round cell	t(12;16)(q13;p11), t(12;22)(q13;q12)	*FUS-DDIT3*
	t(12;22)(q13;q12)	*EWSR1-DDIT3*
Pleomorphic	Complex changes, multiple karyotypes	
Low-grade fibromyxoid sarcoma	t(7;16)(q33;p11)	*FUS-CREB3L2*
		FUS-CREB3L1 (in a small number of cases)
Malignant rhabdoid tumor	Deletion of 22q	*INI1* inactivation
Malignant peripheral nerve sheath tumor	Complex changes	*NF1* inactivation, *INK4A* deletion
Myxofibrosarcoma	Ring form of chromosome 12, complex changes	
Myoxinflammatory fibroblastic sarcoma	t(1;10)(p22;q24), t(2;6)(q31;p21.3)	
Rhabdomyosarcoma		
Embryonal	Trisomies 2q, 8, and 20	Loss of heterozygosity at 11p15
Alveolar	t(1;13)(p36;q14)	*PAX7-FKHR*
	t(2;13)(q35;q14)	*PAX3-FKHR*
Synovial sarcoma	t(X;18)(p11;q11)	*SS18-SSX1, SS18-SSX2*
		SS18-SSX4 (very rarely)
	t(X;20)(p11;q13)	*SS18L1-SSX1*

TRANSLOCATIONS AND OTHER GENETIC ANOMALIES IN SARCOMAS

Genetic Findings in Benign and Intermediate Soft Tissue Tumors

Histologic Type	Translocation of Rearrangement	Fusion Gene or Other Feature
Adipose tumors		
Lipoma	t(3;12)(q27-28;q15), *HMGA2* rearrangements at 12q15	*HMGA2-LPP* fusion
Spindle cell and pleomorphic lipoma	Loss of 13q12, 16q13, polysomy of 12	*RB*/13q14 allelic loss
Hibernoma	11q13 rearrangements	
Lipoblastoma	8q11-13 rearrangements	*PLAG1-HAS2*
Chondroid lipoma	t(11;16)(q13;p12-13)	
Cellular angiofibroma	Loss at 13q	*RB*/13q14 allelic loss
Chondroma of soft tissue	*HMGA2* rearrangements	
Desmoplastic fibroblastoma	t(2;11)(q31;q12)	
Fibroma of tendon sheath	t(2;11)(q31-32;q12)	
Fibromatosis		
Sporadic deep		*CTNNB1* (β-catenin gene) mutations
In familial adenomatous polyposis		Germline *APC* inactivating mutations
Leiomyoma		
Cutaneous hereditary	1q42.3-q43 rearrangements	*MCUL-1* mutations (fumarate hydratase)
Uterine	*HMGA2* rearrangements	
Nerve sheath tumors		
Neurofibroma	9p21-22 rearrangements	*NF1* deletion (17q11) in neurofibromatosis type 1
Perineurioma	22q11.2-12 rearrangements	*NF2* loss
Sclerosing perineurioma	t(2;10)(p23;q24), monosomy 10	
Schwannoma	Changes in 22, 7, X, Y	*NF2* mutations in neurofibromatosis type 2
Mammary-type myofibroblastoma	Partial monosomy of 13q and 16q	Allelic loss at *RB*/13q14 and *FKHR*/13q14
Myoepithelioma	t(1;22)(q23;q12)	*EWSR1-PBX*
	t(19;22)(q13;q12)	*EWSR1-ZNF444*
		EWSR1-POU5F1
Plexiform fibrohistiocytic tumor	t(4;15)(q21;q15)	
Solitary fibrous tumor	12q15 rearrangements	
	t(8;12)(p11.2;q24.3)	
	t(12;17)(q15;q23)	
Tenosynovial giant cell tumor	t(1;2)(2p:13q)	*CSF1-COL6A3*

How and Why translocations arise?

- They are **largely random events** that become fixed through natural selection if they provide a growth advantage to the cell

- Cellular factors that predispose: incorrect DNA repair, less protected chromatin conformation,

Diagnostic Methods

- According to standard chromosome nomenclature, chromosome regions are subdivided into **bands** and at higher resolution **subbands**

- E.g. 12q13 indicates chromosome 12, the long arm (q) region 1, band 3

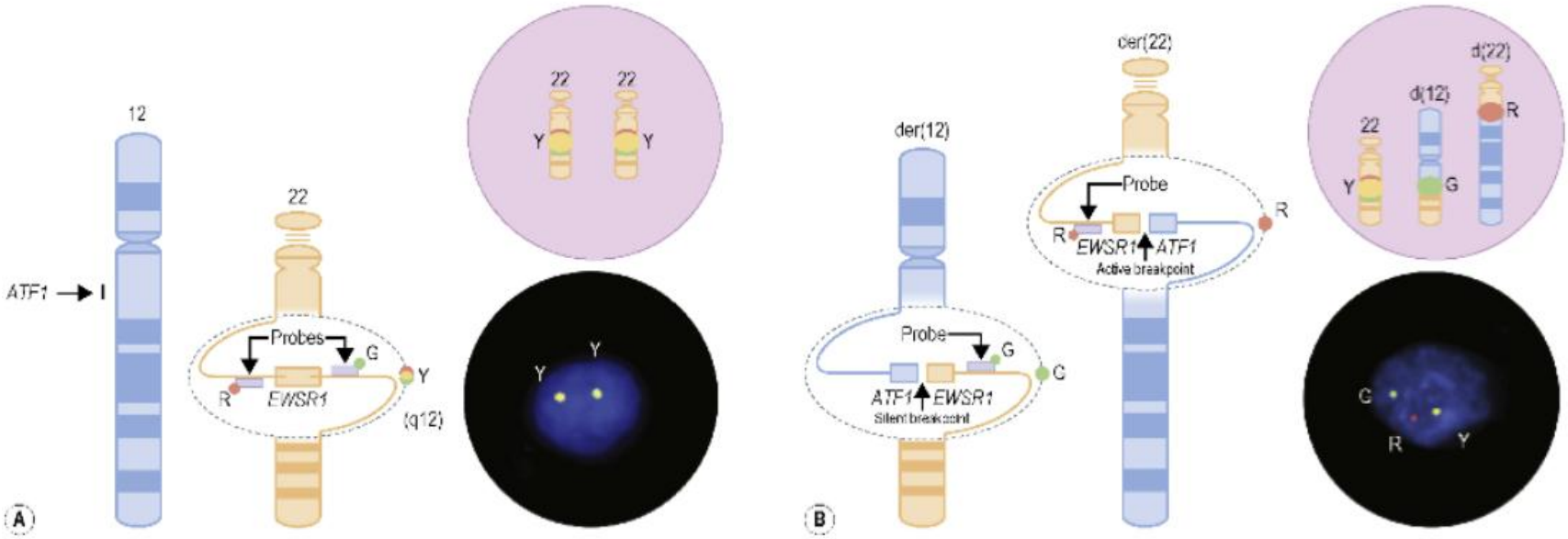
Chromosome

- **Numeric** or **structural**
  - Numeric changes include either gain (+) or loss (-) of chromosomes (e.g +7 indicates an extra copy of chromosome 7 or trisomy 7)
  - Chromosomes involved in structural change is specified in parentheses, directly following the symbol identifying the type of rearrangement [t(12;16)(q13;p11)]

Fluorescence In situ Hybridization (FISH)

- **Break-apart probe** consists of a pair of probes that flank a gene locus of interest, labeled in two different fluorochromes

- Especially useful for detecting chromosomal rearrangements of genes for which translocation partners are variable


12
ATF1
22
Probes
G
R
EWSR1
Y
(q12)
22
22
Y
Y
Y
Y
A
der(22)
der(12)
Probe
R
EWSR1
ATF1
Active breakpoint
R
Probe
G
C
ATF1
EWSR1
Silent breakpoint
B
der(22)
der(12)
22
R
Y
G
C
R
Y

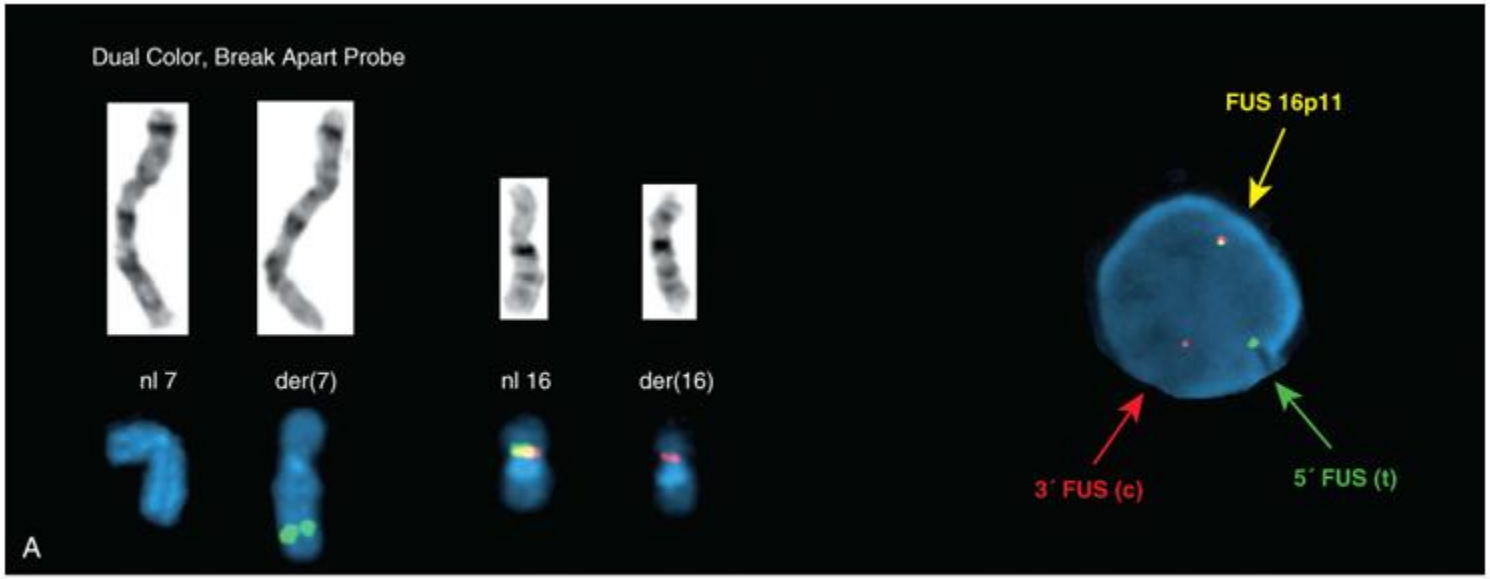


FIGURE 4-2A. Two types of FISH assay designs for translocation detection. nl, normal; c, centromeric; der, derivative chromosome; t, telomeric.

FISH

- Well suited to identify submicroscopic changes and define cryptic and often complex chromosomal rearrangements
- Dividing cells are not longer a prerequisite (**non-dividing/interphase nuclei**)
- Rely upon an a priori knowledge of the targeted aberration and appropriate rational probe design

Limitations

- Break-apart probes are optimal for the detection of rearrangements **consistently involving a single chromosomal region** (e.g., EWSR1) in concert with several different chromosomal partners

- This design **cannot identify the translocation partner**.

Reverse-Transcriptase-Polymerase Chain Reactions (RT-PCR)

- **Detects specific fusion RNA** transcribed from the fusion gene by using forward and reverse primers bracketing the fusion point in the RNA

- To be used in the PCR reaction, the RNA must be converted into complimentary DNA (cDNA) using the enzyme RT

RT-PCR

- Especially powerful because the splicing of the transcripts encoded by fusion genes typically results in very consistent fusion points

- However, RT-PCR, as an RNA-based assay, is susceptible to failure because of **poor RNA quality.**

- It is also susceptible to false-positives because of PCR **cross-contamination**

Examples

- Undifferentiated small round cell sarcomas
- Confirmation of alveolar subtype in rhabdomyosarcoma (as a prognostic factor)
- Monophasic synovial sarcoma from other spindle cell sarcomas
- Confirmation of typical sarcomas in an unusual demographic setting or with unusual histologic/immunocytochemical features.

Applications of Next-Generation Sequencing to Gene fusion discovery or diagnosis

- Discovery of:
  - **BCOR-CCNB3** gene fusion of **small round blue cell tumors** lacking an EWSR1-ETS family translocation
  - **WWTR1-CAMTA1** gene fusion generated by t(1;3)(p36;q25) translocation in **epithelioid hemangioendothelioma**
  - Highly recurrent **NAB2-STAT6** fusion in **solitary fibrous tumor**

Immunohistochemical Markers of genetic alterations

- Many translocations can be converted into immunohistochemistry (IHC) assays based on
  - the phenomenon of discordance in the expression levels of the amino- and carboxy-terminal ends of the product of gene B in tumors with A-B gene fusions
  - the markedly aberrant expression of the carboxy-terminal encoded by gene B in the context of these fusion proteins
  - their expression in aberrant cell types or aberrant cellular compartments

- In sarcomas, this approach has been applied to the detection of the
  - **EWSR1-WT1** protein (desmoplastic small round cell tumor)
  - **ASPSCR1-TFE3** protein (alveolar soft part sarcoma)
  - **EWSR1-FLI1** or **EWSR1-ERG** protein (Ewing)
  - **ALK** fusion proteins (IMT)

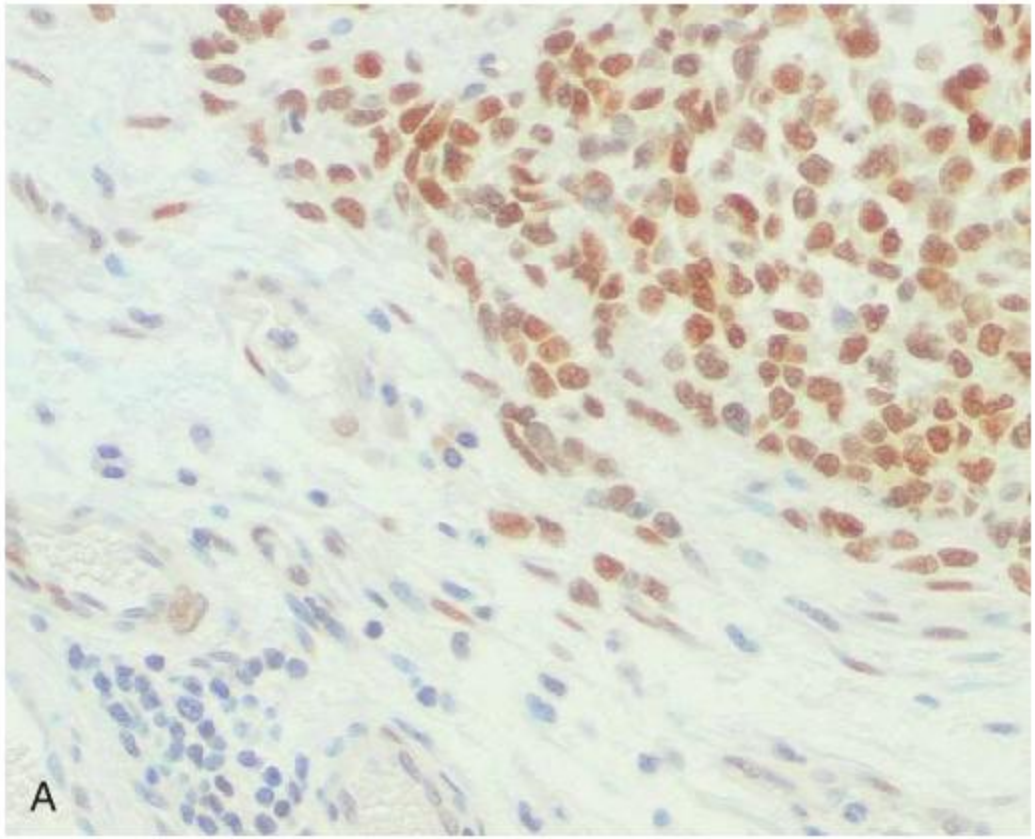


FIGURE 4-3A. Two examples of IHC detection of translocation fusion proteins. (A) The detection of the EWSR1-WT1 protein using antibody to the WT1 carboxy-terminal. (B) The detection of the ASPSCR1-TFE3 protein using antibody to a portion of TFE3 included in the fusion protein.

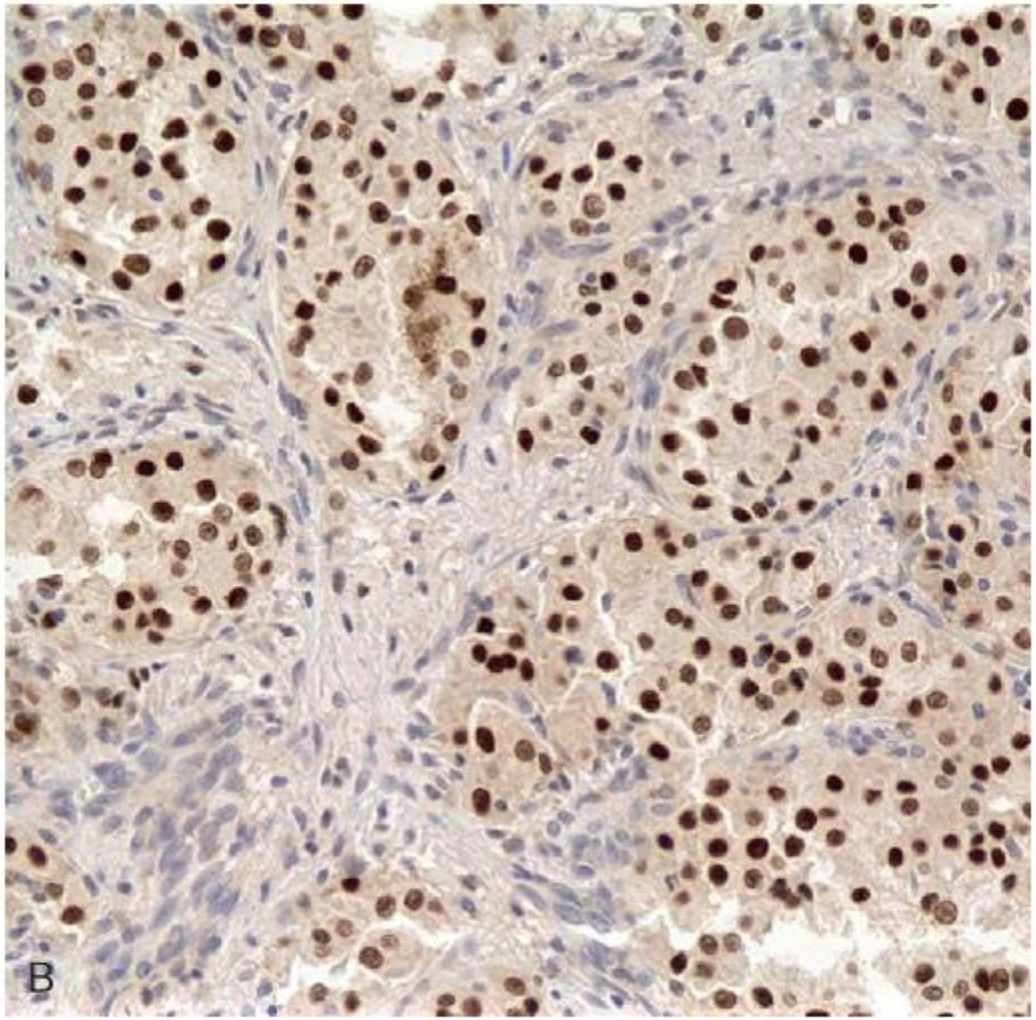


FIGURE 4-3B. Two examples of IHC detection of translocation fusion proteins. (A) The detection of the EWSR1-WT1 protein using antibody to the WT1 carboxy-terminal. (B) The detection of the ASPSCR1-TFE3 protein using antibody to a portion of TFE3 included in the fusion protein.

IHC for other genetic alterations other than translocations

- **KIT (and PDGFRA)** mutations in gastrointestinal stromal tumors (GISTs)
- **Coamplification of MDM2 and CDK4** in well-differentiated and dedifferentiated liposarcomas caused by 12q amplification
- **Mutations involving the WNT pathway** (in APC or in the beta-catenin gene) are characteristic of (but not specific for) aggressive desmoid-type fibromatosis
- **INI1(SMARCB1) loss** in malignant rhabdoid tumor and epithelioid sarcomas

Conclusion

- The specificity of chromosomal translocations in sarcomas is remarkable
- The specificity of sarcoma gene fusions for certain tumor types may reflect a dynamic relationship with the **cellular environment**
- In this model, the gene fusion is oncogenic in a specific **susceptible cell type at a particular developmental stage**, and, in turn, the gene fusion may then modify the phenotype of the susceptible cell

- Aberrant transcription factors may be tolerated and are transforming in only a very specific cell type

- Chimeric transcription factors may have a **strong effect on cell lineage markers**, in effect, redirecting differentiation (reprogramming)

- The phenotype of the precursor cells may be difficult to infer from the phenotype of the translocation sarcoma
- **The precursor cells for a given type of translocation sarcoma may not be exactly identical from case to case.**
  - introduction of the EWSR1-FLI1 gene into neuroblastoma cells or embryonal rhabdomyosarcoma cells can shift their differentiation program to that of ES/PNET
  - FUS-DDIT3 can induce fibrosarcoma cells to display features of liposarcoma

By Diego Rivera
1886-1954