Molecular Pathology of Soft Tissue Tumors

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- Uncommon tumors:
  - ~1% of all cancers (all ages)
  - ~15% of cancers in childhood and adolescence (<20 y/o)

- Lifetime risk:
  - 1 out of 350 people

- Annual incidence, all ages (U.S. SEER data 2005-2009): – Soft tissue and bone: 12,000 - 15,000 new cases/year
  - Undifferentiated pleomorphic sarcoma 30.2%
  - Liposarcoma 16.5%
  - Leiomyosarcoma 13.7%
  - Fibrosarcoma 6.3%
  - Synovial sarcoma 5%
  - Rhabdomyosarcoma 4.3%
  - Dermatofibrosarcoma protuberans 3.2%
  - Myxosarcoma 0.8%
  - Alveolar soft part sarcoma 0.5%
  - Other 19.5%

• 850-900 new cases/year in children and adolescents (<20 y/o) – ~50% are rhabdomyosarcoma

- Sarcomahelp.org SEER.cancer.gov

- Similar “cancer” criteria for sarcoma.....**but**

- Multistep tumorigenesis seen in epithelial neoplasia (carcinomagenesis) has been more difficult to document

– Example: translocation associated sarcomas
• No pre-neoplastic cell identified
• Translocation may be the only genetic alteration; sufficient for the development of mesenchymal cancer

- Cell type specificity appears to be important in sarcomagenesis

– Same translocation may be found in different tumors

Classification of cancer genes

- Mechanistic classification

– Tumor suppressor genes
• Deactivation: deactivating mutation, deletion, or reduced expression

– Oncogenes
• Activation: activating mutation or amplification

– Caretaker genes
• More likely to develop mutation(s) in tumor suppressor genes or oncogenes

- Functional classification

– Protein kinases
• Can function as oncogenes or tumor suppressor genes

– Transcription factors
• Can function as oncogenes or tumor suppressor genes

– DNA maintenance and repair proteins
• Caretaker genes

1. Variable & complex abnormal karyotype 50%
   - Spindle cell/pleomorphic sarcoma, NOS
   - Leiomyosarcoma
   - Myxofibrosarcoma
   - Pleomorphic liposarcoma
   - Pleomorphic rhabdomyosarcoma
   - Other
2. Reciprocal translocations 15-30%
   - Ewing sarcoma (*EWSR1-FLI1*)
   - Synovial sarcoma (*SS18-SSX*)
   - Other
3. Specific mutations
   - Gastrointestinal stromal tumors (*KIT* & *PDGFRA* activating mutations)
   - Other
4. Amplifications
   - Well-differentiated liposarcoma/atypical lipomatous tumor (12q13-15 ring chromosome; *MDM2*)
   - Other

Why test?

- Potential type(s) of information obtained:

– **Diagnostic**: Aid in rendering a morphologic diagnosis
– **Prognostic**: Educated guess at a tumor's behavior without the influence of treatment

– **Predictive**: Response of tumor to therapy

Method	Advantages	Disadvantages
Cytogenetics DNA	Global view Primary and secondary abnormalities identified Does not require knowledge of abnormality or diagnosis May detect abnormalities not seen by FISH or PCR	Requires fresh tissue (dividing cells) Low resolution Cryptic rearrangements Lower sensitivity Slower TAT
FISH DNA or RNA	More targeted view Requires prior knowledge of abnormality or diagnosis Diagnostically specific and sensitive Moderate resolution Moderate analytic sensitivity Multiple tissue types can be used FFPE, frozen, cytology or cultured cells (FISH or iFISH) Can localize abnormality to specific cells Faster TAT	Need for fluorescence microscope Signals fade Does not work on decal tissue
RT-PCR (reverse transcriptase) RNA	High resolution (very targeted view) High sensitivity and specificity, and quantifiable (MRD) Multiplexing possible Can use FFPE, frozen sections, cytology, or fresh Faster TAT	Requires knowledge of abnormality Does not work on decal tissue FFPE may have degraded RNA PCR inhibitors
IHC Protein	Can use FFPE, frozen sections or cytology Morphologic correlation Rapid TAT Relatively inexpensive Mutation specific antibodies available	Interlab variablility
NGS DNA or RNA	High throughput (huge multiplex capability) High resolution (individual nucleotide level)	Cost of equipment (decreasing) Need and cost of bioinformatics

Morphological Groups

- round cell
- spindle
- epithelioid/polygonal
- pleomorphic
- adipocytic
- myxoid
- giant cell
- others

Cytogenetics and Molecular Genetics

(1) soft-tissue neoplasms associated with complex karyotypes

(2) soft-tissue neoplasms characterized by recurrent chromosomal structural abnormalities, gene amplification, mutations, or loss of heterozygosity

Round Cell Tumors

- DD in small round blue cell tumors
- Most of this group are associated with specific translocations

Round cell tumors

Ewing Sarcoma	t(11;22)(q24;q12)
Ewing-like sarcomas	t(4:19)(q35;q13) t(10;19)(q26;q13) t(X;19)(q13;q13.3) X chromosome paracentric inversion
ARMS	t(2;13)(q35;q14) t(1;13) (p36;q14)
Desmoplastic small round cell tumor	t(11;22)(p13;q12
EMC	t(9:22) (q22;q12) t(9;17)(q22;q11)

Ewing Sarcoma

- Second most common in children and young adults
- 10-20% occur at extraskeletal sites
- t(11;22)(q24;q12) in 85% - EWSR (22) / FL11 (11) fusion
- t(21;22)(q22;12)in 5-10% - EWSR1 (22) / ERG (21)
- In rare cases FUS-ERG and FUS-FEV fusion (FUS is similar to EWSR1, part of the TET family)

EWSR1 fusion

- Ewing sarcoma
- myxoid liposarcoma
- angiomatoid fibrous histiocytoma
- myoepithelioma
- myoepithelial carcinoma
- mixed tumor of soft tissue
- clear cell sarcoma of soft tissue
- extraskeletal myxoid chondrosarcoma
- malignant gastrointestinal neuroectodermal tumor (previously referred to as clear cell sarcoma-like gastrointestinal tumor)
- low-grade fibromyxoid sarcoma,
- desmoplastic small round cell tumor

Group of small round cell sarcomas with features similar to Ewing sarcoma

- rearrangements of *EWSR1* with non-*ETS* gene partners
- No rearrangement of *EWSR1* or other TET family members
- In small subset - *CIC-DUX4* gene fusion resulting from t(4:19)(q35;q13) or t(10;19)(q26;q13)
- *CIC-FOXO4* gene fusion, t(X;19)(q13;q13.3) in 2 cases
- *BCOR-CCNB3* fusion gene, arising from an X chromosome paracentric inversion (biologically distinct entity within undifferentiated round cell sarcomas)

Practical approach

- RT-PCR
- IHC with cyclin B3

- Unclear classification
- Treatment similar to Ewing sarcoma

Alveolar Rabdomyosarcoma

- Adolescents and young adults
- t(2;13)(q35;q14) occurs in approximately 60% of cases, t(1;13) (p36;q14) occurs in a smaller subset
- Translocations involve FOXO1A (13) and PAX3 (2) or PAX7(1)
- No specific chromosomal abnormality in embryonal rhabdomyosarcoma (identification of t(2;13) or t(1;13) is diagnostically valuable and prognostically significant - *PAX-FOXO1A* fusion status -unfavorable outcome for children with rhabdomyosarcoma)

Desmoplastic small round cell tumor

- Children and young adults
- Widespread abdominal serosal involvement
- t(11;22)(p13;q12) - fusion of *EWSR1* and *WT1*
- identification of the partner gene is warranted for a specific diagnosis

Extraskeleral Myxoid chondrosarcoma

- *NR4A3* rearrangement
- t(9:22) (q22;q12) or, less frequently, t(9;17)(q22;q11)
- Fusion of *NR4A3* (9q22) to either *EWSR1* (22q12) or *TAF15* (17q12)
- IHC is not helpful in the diagnosis of EMC
- EMC and mixed tumor of soft-tissue and myoepithelioma have overlapping histological and IHC features – different treatment
- These two may share EWSR1 rearrangement
- FISH for NR4A3 – ideal test platform

Spindle cell tumors

Synovial Sarcoma	t(X;18)(p11.2;q11.2)
DFSP	supernumerary ring chromosomes unbalanced derivatives of t(17;22)(q22;q13)
low- grade fibromyxoid sarcoma	t(7;16) t(11;16)
Congenital or infantile fibrosarcoma	t(12;15)(p13;q26)
Congenital or infantile spindle cell rhabdomyosarcoma	*NCOA2* arrangements
SFT	*NAB2-STAT6* fusion
GIST	*KIT* exon 9 *KIT* exon 11 *PDGFRA* exon 18

Synovial Sarcoma

- recurrent reciprocal t(X;18)(p11.2;q11.2), which fuses *SYT* (18q11) to 1 of the 3 homologous genes on Xp11 (*SSX1*, *SSX2*, or *SSX4*)

Dermatofibrosarcoma protuberans

- supernumerary ring chromosomes
- unbalanced derivatives of t(17;22)(q22;q13)
- chimeric gene that fuses COL1A1 with PDGFB in both cases
- multiplex RT-PCR or, preferably, FISH
- therapy with imatinib mesylate may be clinically useful

Low- grade fibromyxoid sarcoma

- *FUS-CREB3L2* gene fusion or, less frequently, *FUS-CREB3L1* gene fusion
- t(7;16) or t(11;16)
- *EWSR1-CRE- B3L1* gene fusion (in a small number)

Sclerosing epithelioid fibrosarcoma

- considerable morphological overlap with low-grade fibromyxoid sarcoma
- *EWSR1* gene rearrangements
- a minority of cases exhibits *FUS-CREB3L2* fusions
- Both show mucin 4 expression by IHC
- perhaps part of a disease spectrum

Congenital or infantile fibrosarcoma

- histologically similar to adult fibrosarcoma
- t(12;15)(p13;q26) resulting in the *ETV6-NTRK3* fusion
- histological features may mimic other pediatric spindle cell neoplasms, such as infantile fibromatosis and infantile myofibromatosis or myofibroma
- It is often difficult to cytogenetically identify the *ETV6-NTRK3* fusion, so it is typically detected by FISH or PCR

Congenital or infantile spindle cell rhabdomyosarcoma

- No *PAX3-FOXO1* and *PAX7-FOXO1* fusions
- Present *NCOA2* arrangements

Solitary fibrous tumor

- NAB2-STAT6 fusion
- Fusion variants NAB2ex4-STAT6ex2/3 and NAB2ex6-STAT6ex16/17 have been identified
- NAB2ex4-STAT6ex2/3 - pleuropulmonary SFTs and mostly exhibits a benign behavior
- NAB2ex6-STAT6ex16/17 - deep-seated extrapleural SFTs and has more aggressive behavior
- STAT6 IHC can be a useful adjunct tool in the diagnosis of SFT (generally excepted test – integrative sequencing)
- STAT6 amplification has been described in a small subset of dedifferentiated liposarcomas (potential pitfall, particularly in retroperitoneal masses)

GIST

- *KIT* exon 9
- *KIT* exon 11
- *PDGFRA* exon 18
- *BRAF* V600E mutation was identified in patients with GIST lacking *KIT* and *PDGFRA* mutations
- GIST are typically associated with the Carney triad and Carney–Stratakis syndrome when they show mutations in *SDH*-related genes

Lipomatous tumors

Myxoid/round cell liposarcoma	t(12;16)(q13;p11)
ALT/WDL	supernumerary ring chromosomes giant-marker chromosomes corresponding to amplification of the 12q13-15 band MDM2

Myxoid/round cell liposarcoma

- *FUS-DDIT3* chimeric gene due to reciprocal t(12;16)(q13;p11)
- myxoid liposarcomas are sensitive to radiation therapy and select patients receive neoadjuvant therapy

Lipoma VS ALT/WDL

- Differently from normal fat, lipoma exhibits a *HMGA2* translocation
- LT/WDL is likely to recur and carries the risk of dedifferentiation, which results in a poor prognosis depending on the anatomical location
- dedifferentiated liposarcoma with predominant, high-grade dedifferentiated areas may be difficult to discriminate from other high-grade pleomorphic sarcomas
- ALT/WDL – supernumerary ring chromosomes and/or giant-marker chromosomes corresponding to amplification of the 12q13-15 band

Table 1. — MDM2-Positive Soft-Tissue Tumors

Tumor Type	MDM2 by FISH	MDM2 by IHC
Lipoma	–	–
Atypical lipomatous tumor/ well-differentiated liposarcoma	+	+ (nuclear)
Dedifferentiated liposarcoma	+	+ (diffuse, nuclear)
Intimal sarcoma	+	+ (≤ 70%)

FISH = fluorescence in situ hybridization, IHC = immunohistochemistry, MDM2 = mouse double minute 2 homolog.

Tumors of uncertain histogenesis

Clear cell sarcoma	(12;22) (q13;q12) t(2;22)(q32.3;q12)
Alveolar soft-part sarcoma	der(17)t(X;17) (p11;q25)
Soft tissue angiofibroma	t(5;8) (p15;q13)

Clear cell sarcoma

- *EWSR1-ATF1* fusion in more than 90% of cases from a reciprocal t(12;22) (q13;q12)
- *EWSR-CREB1* fusion in small subset - t(2;22)(q32.3;q12)
- Some have BRAF mutations

Alveolar soft-part sarcoma

- recurrent, unbalanced der(17)t(X;17) (p11;q25) involving the fusion of *TFE3* (Xp11) and *ASPSCR1* (17q25)
- Although *ASPSCR1-TFE3* appears specific for alveolar soft-part sarcoma, this same gene fusion has been identified in a small subset of renal cell carcinomas

Soft tissue angiofibroma

- benign, fibrovascular soft-tissue tumor of uncertain cellular origin
- *AHRR-NCOA2* fusion resulting from t(5;8) (p15;q13)
- evaluation of *NCOA2* rearrangements using FISH

Myxoid tumors

Table 2. — Genetics of Myxoid Sarcomas

Tumor Type	Defect	Gene
Myxoma	Activating Gs-α mutations	*GNAS*
Low-grade fibromyxoid sarcoma/hyalinizing spindle cell tumor with giant rosettes	t(7;16)(q33;p11) t(11;16)(p11;p11) t(16;xx)(p11)	*CREB3L2-FUS* *CREB3L1-FUS* *EWSR1-CREB3L1*
Myxoid liposarcoma	t(12;16)(q13;p11) t(12;22)(q13;q12)	*DDIT3-FUS* *DDIT3-EWSR1*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21) t (3;9(q11;q22)	*NR4A3-EWSR1* *NR4A3-TAF2N* *NR4A3-TCF12* *NR4A3-TFG*
Myxofibrosarcoma	None characteristic	None
Myxoinflammatory fibroblastic sarcoma	t(1;10)	*TGFBR3-MGEA5*

Histological 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) t(12;16)(q13;p11) t (2;22)(q33;q12)	*EWSR1-ATF1* *FUS-ATF1* *EWSR1-CREB1*
Clear cell sarcoma	t(12;22)(q13;q12)	*EWSR1-ATF1*
Clear cell sarcoma (GIT)	t (2;22)(q33;q12)	*EWSR1-CREB1*
Dermatofibrosarcoma protuberans	t(17;22)(q21;q13) Ring form of chromosomes 17 and 22	*COL1A1-PDGFB*
Desmoplastic SRCT	t(11;22)(p13;q12)	*EWSR1-WT1*
Epithelioid haemangioendothelioma	t(1;3)(p36.3;q25)	*WWTR1-CAMTA1*
Epithelioid sarcoma	Abnormalities of 22q	INI1 inactivation

Ewing sarcoma/PNET	t(11;22)(q24;q12) t(21;22)(q12;q12) t(2;22)(q33;q12) t(7;22)(p22;q12) t(17;22)(q12;q12) inv(22)(q12;q12)	*EWSR1-FLI1* *EWSR1-ERG* *EWSR1-FEV* *EWSR1-ETV1* *EWSR1-E1AF* *EWSR1-ZSG*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21)	*EWSR1-NR4A3* *TAF1168-NR4A3* *TCF12-NR4A3*
Fibrosarcoma, infantile	t(12;15)(p13;q26) Trisomies 8, 11, 17, and 20	*ETV6-NTRK3*
Inflammatory myofibroblastic tumour	2p23 rearrangement	*ALK* fusions with various genes
Leiomyosarcoma	Deletion of 1p	
Liposarcoma: Well-differentiated Myxoid/round cell Pleomorphic	 Ring form of chromosome 12 t(12;16)(q13;p11) t(12;22)(q13;q12) Complex	 *FUS-DDIT3* *EWSR1-DDIT3*

Low-grade fibromyxoid sarcoma	t(7;16)(q33;p11)	*FUS-CREB3L2* *FUS-CREB3L1* (rare)
Malignant rhabdoid tumour	Deletion of 22q	INI1 inactivation
Myoepithelial tumour of soft tissue	t(19;22)(q13;q12) t(1;22)(q23;q12) t(6;22)(p21;q12)	*EWSR1-ZNF444* *EWSR1-PBX1* *EWSR1-POU5F1*
MPNST	Complex	
Myxofibrosarcoma	Ring form of chromosome 12	
Primary pulmonary myxoid sarcoma	t (2;22)(q33;q12)	*EWSR1-CREB1*
Rhabdomyosarcoma: Embryonal Alveolar	 Trisomies 2q, 8, and 20 t(1;13)(p36;q14) t(2;13)(q35;q14)	 LOH at 11p15 *PAX7-FKHR* *PAX3-FKHR*
Synovial sarcoma	t(X;18)(p11;q11) t(X;20)(p11;q13)	*SS18-SSX1* *SS18-SSX2* *SS18-SSX4* (rare) *SS18L1-SSX1*

Thank you for attention

- **Molecular Pathology of Soft-Tissue Neoplasms and Its Role in Clinical Practice** *Evita B. Henderson-Jackson, MD, and Marilyn M. Bui, MD, PhD*

- Molecular Pathology of Soft Tissue Sarcomas *Jon D. Wilson, MD jon.wilson@nephropath.com (lecture)*

- *The royal college of pathologists* - Standards and datasets for reporting cancers Dataset for histopathology reporting of soft tissue sarcomas. January 2017 Author: Professor Cyril Fisher. Consultant Histopathologist, Sarcoma Unit, Royal Marsden NHS Foundation Trust, London Professor of Tumour Pathology, Institute of Cancer Research, University of London