Molecular Diagnosis in STS

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Diagnosis of sarcoma

- **Conventional**
  - Histology and IHC
  - Clinical and radiologic correlation

**Limitation**
- Unusual locations – internal organs etc.
- Unusual age groups and clinical presentation
- Small biopsy
- Unusual IHC profile
- Histologic mimics - carcinoma
MOLECULAR DIAGNOSIS OF STS

- Crucial for diagnostic histopathology of soft tissue sarcoma.
- Unraveling novel molecular targets for more specific therapeutic approach and drug development “targeted therapy”: ALK, ROS, NTRK etc.
- For publication in good journals
“Three groups” of soft tissue tumors are recognized:

- Tumors with complex karyotypes (multiple loss and gain of chromosomes without recurrent patterns among cases)
- Tumors with specific chromosomal translocation
- Tumors with specific gene mutations or amplifications
1. Tumors with complex karyotypes

- Characterized by pleomorphic/spindle cell morphology.
- Including undifferentiated pleomorphic sarcoma (UPS), pleomorphic liposarcoma, leiomyosarcoma, pleomorphic rhabdomyosarcoma, malignant peripheral nerve sheath tumor (MPNST), fibrosarcoma etc.

Molecular test seems to have limited role for diagnosis.
Tumors with complex karyotypes

Myxofibrosarcoma
Tumors with complex karyotypes

Pleomorphic rhabomyosarcoma
2. Tumors with specific chromosomal translocation

- Approximately one-third of soft tissue tumors
- Uniform round cell or spindle cell morphology
- Unique to a specific tumor type, serving as ideal molecular diagnostic markers.
- Some chimeric proteins may constitute excellent treatment targets such as ALK, ROS1, NTRK etc.
Tumors with specific chromosomal translocation

Synovial sarcoma
Tumors with specific chromosomal translocation

Ewing sarcoma: EWSR1-FLI1
Same types of fusion genes may be found in other soft tissue/bone tumors as well as epithelial tumors or hematologic tumors.
3. Tumors with specific gene mutations or amplifications

- Atypical lipomatous tumor/well differentiated liposarcoma: MDM2 amplification.
- Aggressive fibromatosis: beta-catenin mutation.
- Giant cell tumor: H3F3A and H3F3B mutations.
Tumors with specific gene mutations or amplifications

well differentiated liposarcoma: MDM2 gene amplification
Molecular techniques

- Reverse transcription PCR (RT-PCR):
  - Detection of tumor specific fusion genes
    (Group 2: tumors with specific chromosomal translocation)
    - Specific but may not sensitive
    - Good for fresh frozen tissue.
    - Poor RNA quality in FFPE, and novel fusion variant

50 bp marker

PCR primers for the detection of NAB2/STAT6 fusion gene in solitary fibrous tumor (SFT)
Direct sequencing of the PCR product showed fusion of NAB2 exon 6 with STAT6 exon 17.
Molecular techniques

- Fluorescent in situ hybridization (FISH): break-apart technique
  - More sensitive for limited tissue sample (FFPE)
  - More expensive
  - Does not identify translocation partner
  - Positive Cut-off criteria needs to be validated by laboratories.
Chromosomal translocation will induce separation of red and green probes “Break-apart positive” generally, the cut-off criteria for positive cases is 15% (can be 10%-30% or more)
Green signal = centromere of chromosome 12
Red signal = MDM2 gene
Example of MDM2 amplification in liposarcoma
Molecular techniques

- **Next generation sequencing (NGS)**
  - sensitive and specific
  - excellent for fresh frozen tissue but also possible for FFPE (not for degraded/old FFPE blocks)
  - expensive
  - recommended for difficult cases in which the exact diagnosis is required
  - discovery of new fusion genes
  - Not practical for analysis of a single case since all NGS platforms are designed for batch analysis.
A 12 year-old girl with mediastinal mass in 2012

Round cells with variations in size, prominent nucleoli, moderate amount of cytoplasm
Round cells with variations in size, prominent nucleoli, moderate amount of cytoplasm
IHC study

- CD99: positive
- FLI1: positive
- CK, desmin, MyoD1, CD34, myogenin: negative

Molecular tests:
- Positive for EWSR1 FISH (80% positive cells)
- RT-PCR not performed (not available in that time).

Initial diagnosis in 2012:
- Ewing sarcoma
Retrieval of all cases with EWSR1 positive for RT-PCR study in 2018

- Additional RT-PCR:
  - Negative for EWSR1-FLI1, EWRS1-ERG
  - Positive for EWSR-ATF1 fusion transcript.

- Final diagnosis in 2018:
  - Clear cell sarcoma of mediastinum.
EWSR1 rearrangements in sarcoma

- Desmoplastic round cell tumor (DSRT) (EWSR1-WT1)
- Extraskeletal myxoid chondrosarcoma (EWSR1-NR4A3)
- Myxoid liposarcoma (EWSR1-DDIT3)
- Clear cell sarcoma (EWSR1-ATF1)
- Angiomatoid fibrous histiocytoma (EWSR1-CREB1)
- Myoepithelial tumor (EWSR1-ATF1 etc.)
- many recently discovered novel sarcomas.
25 year-old middle east woman with ovarian mass

IHC result is compatible with Ewing sarcoma
Molecular test

- EWSR1 FISH: positive 80%
- RT-PCR: negative for EWSR1-FLI1, EWSR1-ERG

Final diagnosis:
- Round cell sarcoma with EWSR1 gene rearrangement.

Ewing sarcoma ??
Comprehensive genomic testing

- Send to Foundation medicine:
  - EWSR1-\textbf{FEV} fusion gene
    (less than 1\% of Ewing sarcoma)

Final diagnosis:
- Ewing sarcoma of ovary
Ewing sarcoma

- “**TET-ETS**” fusion-driven malignancy
- **TET** family of gene: EWSR1, FUS, TAF15
- **ETS** family of gene: FLI1, ERG, ETV1, ETV4, FEV

Almost 99% of Ewing sarcomas carry translocation involving the EWSR1 gene.

- 85% of cases have the classic t(11;22): EWSR1-FLI1
- 10% of cases have t(21;22) : EWSR1-ERG
- Less than 1% of cases have t(2;22) : EWSR1-FEV
- Other less than 1% : non EWSR1-ETS fusions (FUS-ERG)
Ewing-like sarcoma and undifferentiated round cell sarcoma

However, these tumors are very rare in routine practice. Majority are treated as Ewing sarcoma. Clinical response varies according to certain tumor types.
Diagnosis of Ewing sarcoma

- Histology and IHC: majority of cases can be excluded and correctly diagnosed: occasional potential pitfalls
- Histology, IHC, and EWSR1 FISH: better specificity
  - FISH+: exclude other tumors with EWRS1 rearrangement
- Histology, IHC, EWSR1 FISH and RT-PCR: best combination
  - FISH+/RT-PCR+ = definite diagnosis
  - FISH+/RT-PCR- = ???
  - FISH+/RT-PCR- = ??
EWSR1 FISH +/ RT-PCR -

- Sensitivity of RT-PCR, rare/novel fusion variant, poor RNA quality in FFPE etc.
- Should be diagnosed as “Ewing family of tumor/ Ewing sarcoma” for cases with compatible histology and IHC
- Exclude others known tumor with EWSR1 rearrangement
- Optional test: send for NGS
  - Result can be known sarcoma/ novel sarcoma/ uncategorized genetic pattern
  - May be useful for decision making or may not
  - Some cases may fail NGS due to limited tissue amount/ quality
Take-home messages

- Fluorescence in situ hybridisation and reverse-transcriptase PCR on paraffin-embedded tissue are practical and effective ancillary tools in the diagnosis of soft tissue sarcomas.
- The use of both techniques is optimal for maximising the detection rate of translocation-positive sarcomas.
- Results of molecular investigations must be interpreted in conjunction with histology, other ancillary investigations and the complete clinical picture.
Round cell tumor with EWRS1 FISH negative
“Undifferentiated round cell sarcoma”

44-year-old man with forearm tumor in 2011

17-year-old woman with thigh tumor in 2015
Result from next generation sequencing (NGS)

- Recently in 2018, this 2 cases are retrieved for study by NGS.
- The NGS results show CIC-DUX4 fusion transcripts in both cases.
- Final diagnosis: CIC-DUX4 fusion round cell sarcoma

Up to 2/3 of EWRS1 FISH negative cases may be CIC-DUX4 in one study
CIC-DUX4 sarcoma

- More aggressive than Ewing sarcoma.
- Not response well to chemotherapy for Ewing sarcoma
- Poor prognosis
- Wide range of age groups, various locations
Round cell tumor with EWRS1 FISH negative
“Undifferentiated round cell sarcoma”

14-year-old boy with bone tumor at femur

Mixed round and spindle cell tumor

12-year-old boy with soft tissue mass at back
Summary of BCOR-CCNB3 sarcoma tested in Ramathibodi Hospital
Total: 4 cases
All male children age below 15
bone tumor 3 cases, soft tissue 1 case

Better prognosis than Ewing sarcoma in some studies.
Novel entities of round cell sarcomas

- NGS testing reveals numerous novel sarcomas with novel fusion genes other than Ewing sarcoma
- How to classify ???
Molecular classification of round cell sarcoma by gene expression profile

The numbers of novel sarcoma and gene fusion are increasing. These tumors are clearly different from Ewing sarcoma. Specific treatment may be needed for each tumor type.
Spindle cell sarcomas in infant and children

- Infantile fibrosarcoma (IFS):
  - <2 years
  - ETV6-NTRK3 fusion in 2/3 of cases

- How about genetic alteration of ETV6-NTRK3 negative cases or IFS-like sarcoma in older children ??
- Recent findings reveal many novel tumor types
• **Infant group**: 50% BCOR internal tandem duplication, or YWHAE-NUTM2B/E fusion
• Similar genetic to clear cell sarcoma of the kidney
• Variable histology and IHC profile.
### Recurrent BRAF Gene Fusions in a Subset of Pediatric Spindle Cell Sarcomas

*Expanding the Genetic Spectrum of Tumors With Overlapping Features With Infantile Fibrosarcoma*

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<th>Sex</th>
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<th>S100</th>
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<td>Focal+</td>
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<tr>
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<td>M</td>
<td>Thigh</td>
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<td>Rare cells</td>
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<td>+</td>
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<td>–</td>
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</table>

*By targeted RNA sequencing.
†By FISH.
‡By FoundationOne, Foundation Medicine.
§By whole transcriptome sequencing.

F indicates female; IHC, immunohistochemistry; M, male; NA, not available; RP, retroperitoneum.
Paediatric and adult soft tissue sarcomas with *NTRK1* gene fusions: a subset of spindle cell sarcomas unified by a prominent myopericytic/haemangiopericytic pattern

*Journal of Pathology*

J Pathol 2016; 238: 700–710

**TFG-MET** fusion in an infantile spindle cell sarcoma with neural features

IFS-like sarcoma in children

- Diverse groups of sarcomas with different genetic alterations.
- Can be costly to be tested with NGS
- Despite of advanced technology, many cases still have unknown genetic alterations
- Some tumors with NTRK 1-3 fusion may be treated by targeted therapy
Antibody of detection of tumors with NTRK1, 2, 3 fusions

Roche launches first IVD pan-TRK immunohistochemistry assay
The VENTANA pan-TRK (EPR17341) Assay[1] is the first assay of its type to detect tropomyosin receptor kinase (TRK) with anticipated use across multiple solid tumor types (carcinoma and sarcoma)
12 year-old boy with large intracranial tumor

Mesenchymal tumor, not neuronal tumor
12 year-old boy with large intracranial tumor

- Negative for CD34, STAT6 (not SFT), EMA, S100
- Positive for NUTM1, BCOR, TLE1
- Negative for RT-PCR for SYT-SSX fusion
NUTM1 fusion tumors other than NUT midline carcinoma (BRD4-NUTM1) can be found in various locations with various gene fusion partners. All cases are positive to NUTM1 antibody.
Diverse histology of NUTM1 tumors other than NUT midline carcinoma
12 year-old boy with large intracranial tumor

- Might be NUTM1 fusion tumor
- Pending for confirmation by RT-PCR
- To be continued............
34 year-old man with ureteric tumor

Polypoid mass projecting into ureteric lumen
Uniform round cell and spindle cell morphology
- Negative to all IHC markers except vimentin = sarcoma, not epithelial tumor  
- Negative to RT-PCR for Ewing sarcoma, synovial sarcoma, alveolar rhabdomyosarcoma etc.  
- Excellent response to chemotherapy: 100% tumor necrosis  
- Novel sarcoma or known rare sarcoma?
Comprehensive genomic testing

- Send to Dovetail genomics, LLC
- Multiple chromosomal deletions, duplications, translocations

“Tumors with complex karyotypes”
(despite of uniform cell morphology)

- By histology + location + genetic = unique sarcoma without specific genetic alteration pattern
12-year-old girl with shoulder mass

- Sarcoma with uniform cell morphology
- No specific IHC markers, negative to FISH, RT-PCR
- Partial response to treatment + lung metastasis
Comprehensive genomic testing

• Send to Dovetail genomics, LLC
• t(3;7) and segmental duplication of chr 19

“Tumors with specific chromosomal translocation”
(consistent with uniform cell morphology)

• novel sarcoma: pending for analysis of t(3;7)
  : to be continued.....
Fusion genes and prognosis

- Alveolar rhabdomyosarcoma
  PAX7 may be less aggressive than PAX3 fusion
  (PAX3 fusion is the more common)

- Inflammatory myofibroblastic tumor (IMT)
  RANBP2-ALK, ALK negative cases may have worse prognosis
• The presentation was supported by research grant from Ramathibodi Comprehensive Cancer Center.
Thank you for your attention