Molecular Testing versus Morphology in Sarcomas: What is Left to Pathologists?

Andrew L. Folpe, M.D.
Professor of Laboratory Medicine and Pathology
Mayo Clinic, Rochester, MN
folpe.andrew@mayo.edu
The “Dinosaur Talk”

• Time-honored tradition in Surgical Pathology

• Fixture of seminars on “cutting edge” technologies at international meetings

• Follows lectures by “up and coming” young pathologists illustrating the exciting impact of the latest advances in ancillary testing

• An aged and decrepit senior pathologist attempts to justify the continued use of whatever it is that they still remember from when they trained back in the Dark Ages

• Electron microscopy is often involved
Since I am basically a “glass pusher”, let’s look at some glass and see if there might still be a role for morphology in the diagnosis of soft tissue tumors.
Case 1

• A 27-year-old woman presented to another academic medical center with a submucosal laryngeal mass

• Immunohistochemical and molecular genetic studies were performed

• By report, FISH was positive for SS18 rearrangement

• The patient was referred to Mayo Clinic for laryngectomy

• Per routine protocol, the outside slides were re-reviewed at Mayo Clinic on a general surgical pathology service, and the outside diagnosis of synovial sarcoma was confirmed
Case 1 continued

- The surgeon was able to perform a subtotal laryngectomy
- The specimen was sent for frozen section evaluation
- I reported the margins as negative, and deferred the final diagnosis
Monophasic synovial sarcoma
Solitary fibrous tumor
Diagnosis

• Solitary fibrous tumor, with low risk for aggressive behavior, completely resected

• FISH for SS18 rearrangement was repeated at Mayo Clinic, and was negative
Case 2

• A 68-year-old man, with a history of multiple sun exposure-related skin tumors, presented to an outside academic medical center with a mass involving the parotid gland

• By immunohistochemistry, the tumor was positive for S100 protein and SOX10, and negative for HMB45 and Melan-A

• FISH for EWSR1 rearrangement was reported to be positive

• The case was referred to me in consultation as a “clear cell sarcoma”
Clear cell sarcoma

Melan A
Spindle cell melanoma
Diagnosis

- Metastatic spindle cell malignant melanoma
- FISH for *EWSR1*, performed at Mayo Clinic, was negative
False Positive FISH/ RT-PCR Results

• Deletion of nearby genes (e.g., misinterpretation of *SMARCB1* loss as *EWSR1* rearrangement)

• Inappropriately low cutoff thresholds for scoring “positive” break apart results

• Misinterpretation of aneuploidy with whole/partial chromosome loss

• Poor technique

• Human error

• Contamination (RT-PCR)
Case 3

- A 56-year-old woman presented to Mayo Clinic Arizona with appendicitis
- In the course of her work-up she was found to have multiple pulmonary nodules
- The MCA pathologists favored the diagnosis of metastatic extraskeletal myxoid chondrosarcoma
- The case was sent to Mayo Clinic Rochester for *NR4A3* FISH
NR4A3: Negative
Unexpected Negative FISH/ RT-PCR Results

- Small percentage of neoplastic cells in tissue
- Molecular heterogeneity
- Genetic promiscuity
- Rare variant fusions
- Your morphologic impression is wrong
Molecular Heterogeneity

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Fusion Partner</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;22)(q24;q12)</td>
<td>EWSR1-FLI1</td>
<td>95%</td>
</tr>
<tr>
<td>t(21;22)(q22;q12)</td>
<td>EWSR1-ERG</td>
<td>5%</td>
</tr>
<tr>
<td>t(7;22)(p22;q12)</td>
<td>EWSR1-ETV1</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(2;22)(q33;q12)</td>
<td>EWSR1-E1AF</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(1;22)(q42;q12)</td>
<td>EWSR1-?</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(2;22)(q33;q12)</td>
<td>EWSR1-FEV</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(17;22)(q12;q12)</td>
<td>EWSR1-ETV4</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(16;21)(p11;q22)</td>
<td>FUS-ERG</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

Ewing sarcoma
Genetic Promiscuity

Tumors with *EWSR1* Rearrangements

- Ewing sarcoma: *EWSR1-FLI1*, *EWSR1-ERG*, *EWSR1-FEV*, others
- EMC: *EWSR1-NR4A3*
- Myxoid LPS: *EWSR1-DDIT3*
- CCS: *EWSR1-ATF1*, *EWSR1-CREB1*
- DSRCT: *EWSR1-WT1*
- A(M)FH: *EWSR1-ATF1*, *EWSR1-CREB1*
- SEF: *EWSR1-CREB3L1*
- Others

Tumors with *FUS* Rearrangements

- Myxoid LPS: *FUS-DDIT3*
- A(M)FH: *FUS-ATF1*, *FUS-CREB1*
- Ewing sarcoma: *FUS-ERG*, *FUS-FLI1*, others
- LGFMS and hybrid LGFMS/SEF: *FUS-CREB3L2*
- Others
Rare Variant Fusions

- $t(X;18)(p11.23;q11)$ ($SS18-SSX1$): 65%
- $t(X;18)(p11.21;q11)$ ($SS18-SSX2$): 35%
- $t(X;18)(p11;q11)$ ($SS18-SSX4$): <1%
- $t(X;20)(p11;q13.3)$ ($SS18L1-SSX1$): <1%

Synovial Sarcoma
Epithelioid hemangioendothelioma
Diagnosis

- Metastatic epithelioid hemangioendothelioma
- The primary tumor was subsequently identified in the femur
“OK- that’s all well and good. But won’t next-generation sequencing solve all of these problems?”
# Fusions! Fusions Everywhere!

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Fusions Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical Lipomatous Tumor</td>
<td>FRS2-NUP107, OSBP1L8, MDM2, PTCH1-LINC00476, R3CH1-NOTCH2</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>FN1-COL1A1, COL1A2-FN1</td>
</tr>
<tr>
<td>Chordoma</td>
<td>MIR3606-COL3A1</td>
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<tr>
<td>Dedifferentiated Liposarcoma</td>
<td>HMG2A-MYF5, NOTCH2-ILF2, RBPMS-FDFT1, RPSAP52-HMG2A, TAF3-UPF2, SRGAP1-NT5C2, MDM2-RA2B1, MDM2-FBXL14, POE10A-HMG2A, RPSAP52-HMG2A, LGR5-CDK7, TEPL1-CDK4, HMG2A-TSPAN8, KITLG-SLC26A10, PER1-MAFK, NFIX, B4GALNT1</td>
</tr>
<tr>
<td>Dermal-type Fibromatosis</td>
<td>COL1A1-FN1, MIR3606-COL3A1</td>
</tr>
<tr>
<td>Dermatofibrosarcoma Protruberans</td>
<td>COLA1-PDGFB</td>
</tr>
<tr>
<td>Epithelioid Hemangiendothelioma</td>
<td>WWTR1-CAMTA1</td>
</tr>
<tr>
<td>Epithelioid Sarcoma</td>
<td>FBXLI8-RNF216, FN1-IGFBP3</td>
</tr>
<tr>
<td>Ewing's Sarcoma</td>
<td>EWSR1-FU1</td>
</tr>
<tr>
<td>Fibromyxoid Sarcoma</td>
<td>CREB3L2-FUS</td>
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<tr>
<td>Hibernoma</td>
<td>MBDS-RAD51B</td>
</tr>
<tr>
<td>Lipoma</td>
<td>HMG2A-LPP, HMG2A-AHNAK, RPSAP-EBF1, AP3B1-MEGF-3, FN1-COL1A1</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>THBS1-FN1, FN1-WDUB1, TBL1XR1-KCNH6</td>
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<tr>
<td>Myxofibrosarcoma</td>
<td>WHSC1L1-EFIEBP1</td>
</tr>
<tr>
<td>Myxoid Liposarcoma</td>
<td>DDIT3-FUS, FJS-DDIT3</td>
</tr>
<tr>
<td>Myxoma</td>
<td>PRG4-FN1</td>
</tr>
<tr>
<td>Phosphaturic Mesenchymal Tumor</td>
<td>COL1A1-FN1</td>
</tr>
<tr>
<td>High-grade Sarcoma (unspecified)</td>
<td>MBDS-RAD51B</td>
</tr>
<tr>
<td>Solitary Fibrous Tumor</td>
<td>NAB2-STAT6</td>
</tr>
<tr>
<td>STUMP</td>
<td>PUM1-MKL1, HMG2A-RAD51B</td>
</tr>
<tr>
<td>Synovial Sarcoma</td>
<td>SS18-SSX1, SS18-SSX2B</td>
</tr>
<tr>
<td>Well Differentiated Liposarcoma</td>
<td>TRIC-TERT, L2G3-RAD3IP, FRS2-ATF1, C12orf56-HMG2A, HMG2A-SETD1B, RPSAP52-SCARAS, GAS2L3-NUP107, RPSAP52-DCN</td>
</tr>
</tbody>
</table>
Different Tumors-Identical Genetics

Detection and characterization of EWSR1/ATF1 and EWSR1/CREB1 chimeric transcripts in clear cell sarcoma (melanoma of soft parts)

Wei-Lien Wang, Eman Mayordomo, Wennyong Zhang, Vivian S Hernandez, Daniel Tivin, Lisa Garcia, Dina C Lev, Alexander F Lazri, and Dolores Lopez-Terrada

EWSR1-CREB1 and EWSR1-ATF1 Fusion Genes in Angiomatoid Fibrous Histiocytoma

Sabrina Ross, Károly Szuhai, Marije Ijzenga, Hans J. Tanke, Lucia Zanatta, Raf Scoi, Christopher D. M. Fletcher, Angelo P. Dei Tos, and Pancras C.W. Hogendoorn

EWS-CREB1: A Recurrent Variant Fusion in Clear Cell Sarcoma—Association with Gastrointestinal Location and Absence of Melanocytic Differentiation

Cristina R. Antonescu, Khedoudja Nafa, Neil H. Segal, Paola Dal Cin, and Marc Ladanyi

EWSR1-ATF1 Fusion Is a Novel and Consistent Finding in Hyalinizing Clear-Cell Carcinoma of Salivary Gland

Cristina R. Antonescu, Nors Karabali, Lei Zhang, Yun Shao Sung, Raja R. Seethala, Richard C. Jordan, Bayardo Perez-Ordonez, Cherry Hare, Sylvia L. Aas, Iona T. Long, Grace Bradley, Hagen Kleeh, and Ian Weisreb

Primary Pulmonary Myxoid Sarcoma With EWSR1-CREB1 Fusion: A New Tumor Entity

Khin Thway, FRCPath,* Andrew G. Nicholson, DM, FRCPath,† Kay Lawson, MBBS,† David Gonzalez, PhD,‡ Alexandra Rice, FRCPath,† Bonnie Balzer, MD,§ John Swansbury, FRCPath,§ Toon Min, PhD,∥ Lisa Thompson, PhD,∥ Kwame Adu-Poku, FRCPath,∥ Anne Campbell, MD, FRCPath,∥ and Cyril Fisher, MD, DSc, FRCPath*

EWSR1 Fusions With CREB Family Transcription Factors Define a Novel Myxoid Mesenchymal Tumor With Predilection for Intracranial Location

Yu-Chien Kao, MD,* Yun-Shao Sung, MSC,† Lei Zhang, MD,† Chun-Liang Chen, MSC,† Sumathi Vasapuri, MD,‡ Marc K. Rosenblum, MD,‡ and Cristina R. Antonescu, MD†
A 26-year-old man presented with a finger mass. An EWSR1-FLI1 fusion was identified by ARCHER and RT-PCR. Obviously, this is Ewing sarcoma, right?
Positive for CD99, caldesmon, desmin
A 26-year-old woman presented with a shoulder mass. An *EWSR1-ERG* fusion was identified by ARCHER and FISH. Well, this one definitely has to be Ewing sarcoma, right?
So…What is Left to Pathologists?

• A lot of the really hard and clinically relevant things, like deciding if a lesion is benign or malignant, distinction of mesenchymal from non-mesenchymal tumors, margin evaluation etc

• At least 75% of the cases in my consultation archive either lack a known genetic event or do not require genetic evaluation for diagnosis

• Although molecular genetics have had and will continue to have a profound impact on multiple aspects of soft tissue pathology, these tests must be interpreted in the appropriate clinical and morphological context