LOOK-ALIKES IN SPINDLE AND EPITHELIOID TUMORS:
Ultrastructural value and pitfalls in diagnosis

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Dr. Guillermo Herrera declares he has no conflict(s) of interest to disclose.

ANCILLARY DIAGNOSTIC TECHNIQUES

- Immunohistochemistry
- Electron microscopy
- Cytogenetics
- Flow cytometry
- Molecular diagnostics

Arthur Purdy Stout Society of Surgical Pathologists- USCAP

- There Are No Magic Bullets: When Immunostains Can Get You into Trouble
- Sunday, March 5, 2017
  8:30 AM 12:00 PM
  CC Hemisfair 2&3
ISSUES TO BE ADDRESSED USING ELECTRON MICROSCOPY

Epithelial vs mesenchymal DIFFERENTIATION-crucial in the differential diagnosis of these types of tumors
Aberrant expression of antigenic epitopes
Lack of specific immunohistochemical profile and/or molecular imprint
Need to better characterize a rare tumor
CONFIRM A SUSPECTED THOUGH NOT DEFINITIVE DIAGNOSIS OR A RATHER WELL ESTABLISHED DIAGNOSIS TO PROVIDE ADDITIONAL EVIDENCE

THE WORK UP OF A SPINDLE CELL / EPITHELIOID NEOPLASM

Need to develop a differential diagnosis
Judicious use of ancillary diagnostic techniques
What is available and what is desired
Understanding advantages and limitations of techniques available to address the differential diagnosis
Awareness of pitfalls

WHY TO even CONSIDER EM?

- Identifying specific combinations of morphologic findings in the neoplastic cells, rather than staining patterns or other non-morphologic parameters, is generally much more specific and reassuring
EM not only can rule out certain diagnoses but IN A SUBSET OF CASES also suggests or provides definitive evidence for a diagnosis that would not have been considered

VALUE OF ELECTRON MICROSCOPY

MUST BE VIEWED AS MULTIFACETED
EM not only can address the differential diagnosis but may raise new diagnostic possibilities
EM can provide a deeper understanding to the problem
EM provides solid morphologic evidence to substantiate the diagnosis

DIFFERENTIAL DIAGNOSIS OF SOFT TISSUE SARCOMAS WITH EPITHELIOID FEATURES

- Soft tissue sarcomas, especially those poorly differentiated, may mimic a variety of epithelial and EVEN lymphoid neoplasms
- Diagnosis must be approached by using an intelligent selection of ancillary diagnostic techniques
- The most reasonable route to address the differential diagnosis and to make a final diagnosis should be taken
- However, a combination of diagnostic techniques may be needed and should be used in a subset of these cases to arrive to an unequivocal diagnosis

IH IN THE EVALUATION OF SPINDLE / EPITHELIOID TUMORS AND LOOK-ALIKES

- Often not ONE specific antibody to establish cell type and thus address differential diagnosis
- Batteries of stains required commonly resulting in expensive work-ups
- Overlap among IH profiles and confusion with look-alikes
IH and EM in the diagnosis of soft tissue tumors

- 142 consecutive soft tissue sarcomas examined by IH and EM
- Tentative dx in 58 and differential diagnosis in 82
- EM more often contributed to diagnosing tumors than IH (80 vs 65%)

IH MORE OFTEN THAN EM DID NOT PROVIDE INFORMATION TO AID IN THE TYPING


IH AND EM IN THE DIAGNOSIS OF SOFT TISSUE TUMORS

- In general, the value of EM was greater in the more diagnostically challenging cases
- In 47% of the cases in which one of the modalities was non-contributory, the other was helpful in reaching a diagnosis
- Both EM and IH are necessary to properly evaluate soft tissue tumors


Pleural fluid / right pleural mass
60 y/o male

- VIMENTIN, S-100 protein + keratin 1st panel
- Calretinin - 2nd panel
- CK 5/6 - NEGATIVE

FOLLOW UP - MALIGNANT MELANOMA?

Final diagnosis: Metastatic melanoma with aberrant keratin expression
- Melan A and HMB-45 + (3rd panel) - positive

CHALLENGING SITUATIONS

- Differentiation of sarcoma vs carcinoma including sarcomatoid carcinoma
- Differentiation of sarcoma vs sarcomatoid mesothelioma
- Differentiation of pseudomesotheliomatous adenocarcinoma of the lung with florid stromal reaction vs mesothelioma vs sarcoma (i.e. synovial SARCOMA) - biphasic pattern
- Differentiation pleomorphic sarcoma vs poorly diff. carcinoma / melanoma / lymphoma
- Evaluation of fine needle aspirates where sarcoma is in the differential
DIFFERENTIATION OF SARCOMAS FROM CARCINOMAS

- Depending on the situation (site...), the differential diagnosis may vary.
- If sarcomatoid carcinoma is in the differential dx, there are certain areas where this challenge occurs most often: Kidney, bladder, thyroid and upper respiratory tract.
- Proper characterization extremely important to address therapeutic management and defining prognosis.

**LEIOMYOSARCOMA**

Kidney tumor

- Cohesive medium size cells with small processes which interdigitate.
MERKEL CELL CARCINOMA

PERINUCLEAR FILAMENT AGGREGATES

THIGH TUMOR

SARCOMA VS SARCOMATOID MESOTHELIOMAS

Metastatic RCC (clear cell) with sarcomatoid component

SPINDLE CELL / EPITHELIOID TUMORS OF THE PLEURA
SPINDLE CELL TUMORS OF THE PLEURA - DIFFERENTIAL DIAGNOSIS

- Generally challenging
- Similar clinical presentation: localized mass or diffuse pleural thickening, histology: spindle cell tumors with variable pleomorphism and cellularity and immunoreactivity
- Metastatic spindle cell tumors more frequent than malignant mesothelioma
- Often pleural tumors must be evaluated using a multidisciplinary approach to arrive at the correct diagnosis


SARCOMA VS SARCOMATOID MESOTHELIOMA

- Most IH markers of epithelial mesotheliomas are not present in the sarcomatoid variety
- The "positive" mesothelioma markers: podoplanin, calretinin, keratins 5 and 6 and WT1 protein are generally absent or weakly +
- Calretinin most commonly present
- Keratin IH frequently weak, focal or absent and some sarcomas express keratins aberrantly
- No other techniques available for addressing differential diagnosis, except for EM, unless specific molecular markers are searched for

EM IN THE DIFFERENTIAL DIAGNOSIS OF SARCOMATOID MESOTHELIOMA VS SARCOMA

- Variable numbers of fibroblastic cells in MOST areas of tumor examined
- Finding of definitive epithelial differentiation, such as well formed desmosomes and tonofilaments and usually small lumina lined by bushy and tall microvillous borders requires careful and detailed EM analysis
- Absence of specific differentiation along specific mesenchymal cell lines (i.e. smooth or skeletal muscle, schwannian differentiation) is very helpful
- Exclusion of other tumors that may exhibit spindle cell morphology (i.e. melanoma)
DIFFERENTIAL DIAGNOSIS

MESOTHELIOMA / ADENOCARCINOMA VS SYNOVIAL SARCOMA

• DIFFICULT DIFFERENTIAL DIAGNOSIS - SYNVOIAL SARCOMA ONLY RARELY CONSIDERED - need to suspect
• ADENOCARCINOMAS - both 1ary and metastatic - MAY EXPRESS MESOTHELIAL MARKERS and SYNOVIAL SARCOMAS HAVE OVERLAPING IH MARKERS with epithelial tumors

PLEURAL SYNOVIAL SARCOMAS

• X;18 translocation in SS
• Differential diagnosis from malignant mesothelioma requires a high degree of suspicion and complete immuno-morphologic work-up, including molecular analysis in at least some cases
• SS are susceptible to chemotherapy and require such tx while MM are resistant and should not be treated with chemo
SYNOVIAL SARCOMAS
EM DIAGNOSIS

- Apical surface with short and simple microvilli, sometimes with intestinal features forming glands separated from the spindle shaped cells by a well defined basal lamina
- Prominent lysosomes in neoplastic cells
- Tropofilaments (even keratohyaline granules)
- Junctional complexes - including in spindle cell areas
- Amorphous deposits of epithelial mucin in clefts and luminal spaces

( p11.2;q11.2) (X;18)
Translocation X and 18
MOLECULAR ANALYSIS IN SS

- The characteristic X;18 translocation results in the fusion of the SYT gene on chromosome 18 to either SSX1, SSX2, or SSX4 gene on chromosome X.
- Initially felt to be rather specific for SS and present in >90% of SS- CHALLENGED
- Aubry et al confirmed this translocation in 100% of pleural SS (Am J Surg Pathol 25:776-781, 2001)- purely sarcomatoid SS

PLEURAL / MEDIASTINAL SYNOVIAL SARCOMAS

- In equivocal cases, ultrastructural examination coupled with demonstration of the characteristic (X;18) chromosomal translocation in synovial sarcoma may be the only means for establishing a definitive diagnosis.


CHALLENGES IN DIAGNOSIS

Is it really a sarcoma???

- KERATIN, NSE AND DESMIN (+)
- DESMIN (-)
- EWS-WT1 gene fusion +

Final dx: Desmoplastic small cell tumor (DSCT)

20 y/o- abdominal mass
Anti-desmin antibodies in the diagnosis of rhabdomyosarcomas

- Truong, 1990 282 cases+/359 78%
- Leader, 1987 63%
- Myogenin is far more sensitive

PLEOMORPHIC SARCOMAS
DIFFERENTIAL DIAGNOSIS

- NEED TO BE DIFFERENTIATED FROM POORLY DIFFERENTIATED CARCINOMAS AND MELANOMAS; RARELY FROM HIGH GRADE LYMPHOMAS
- INITIAL IMMUNOHISTOCHEMISTRY OR ELECTRON MICROSCOPIC EVALUATION?
BECAUSE IH is more readily available, the former will happen in most institutions

USUAL ANTIBODY BATTERY

- Keratins
- Melan A, HMB-45 and S-100 protein
- Vimentin
- More specific markers such as TTF-1, Hepar-1, PSA, thyroglobulin etc as needed

LUNG, peripheral mass
- Abdominal mass
- KERATIN

CLEAR CELL SARCOMA?

EWS-ATF1 gene fusion
- NOT DETECTED
DIFFICULTIES IN THE EVALUATION OF FINE NEEDLE ASPIRATES IN THE DIFFERENTIAL DIAGNOSIS OF SARCOMAS vs LOOK-ALIKES

- Scanty cellular elements
- No architectural pattern
- Difficulties in selecting the appropriate antibody panel in small samples— not much tissue available
- Problems with IH in FNA samples (background, edge artifact, overstaining)

45 y/o female

FNA Abdominal mass
Lymphoma vs carcinoma vs sarcoma

58 y/o male

FNA Pleura
Sarcoma vs carcinoma

LOOK-ALIKES

EM is a useful technique to separate these look-alikes
- 82 y/o male with history of prior removal of a tumor in the right leg. Now with abdominal mass

- 18 y/o male with a history of sarcoma-left arm- S/P chemotherapy treatment presenting with subcutaneous nodule on his back (approximately 2 cm in diameter)
- CD-99 EQUIVOCAL

- Translocation (11;22) EWS/FLI-1 fusion gene

- 14 y/o girl with history of sarcoma of left femur (? type) who developed a pelvic mass

- 55 y/o male with abdominal mass. No pertinent history elicited
Keratin -, muscle specific actin +, HMB-45 -

Other techniques useful in the diagnosis of spindle / epitheliod tumors and look-alikes in addition to IH and EM

FLOW CYTOMETRY
extremely useful when hematologic conditions are in the differential diagnosis

CYTOGENETICS
certain soft tissue neoplasms have characteristic cytogenetic abnormalities
i.e. synovial sarcomas- TRANSLOCATIONS INVOLVING CHROM X AND 18, Ewing's sarcoma (EWS-Fli1), clear cell sarcoma (EWS-ATF1), myxoid liposarcoma (FUS-CHOP), among others

MOLECULAR DIAGNOSTICS

GUIDELINES FOR EM

• Several pieces of tumor from different areas, avoiding necrotic and hemorrhagic areas should be submitted
• Tissue should be cut in small pieces (1 mm cubes) and promptly fixed in a fixative adequate for EM
• If EM scope or expertise in ultrastructural diagnosis is not available locally, specimen should be sent to regional laboratory

IMPORTANT FACTORS TO BE CONSIDERED WHEN EM IS TO BE USED

AVAILABILITY OF EM LOCALLY
TURN AROUND TIME
TRAINED INDIVIDUALS TO PERFORM EM STUDY
CONSIDER SENDING TO A REGIONAL FACILITY WITH EM SERVICES

PROPER COLLECTION AND FIXATION OF MATERIAL ESSENTIAL TO MAKE SURE THAT THE BEST POSSIBLE EVALUATION IS PERFORMED

MISCONCEPTIONS ABOUT THE ROLE OF EM IN THE EVALUATION OF SOFT TISSUE TUMORS

• IH has made EM obsolete
• Only role of EM is to confirm LM impression
• Only a few sarcomas have specific ultrastructural features
• Very limited value in poorly differentiated tumors
• Sampling of tumors too small to produce meaningful information
• Too slow
• Too expensive

"The problem can often be solved by using one technique or an intelligent combination of a small number of various methods, but rarely by excluding one technique in favor of another..."

Dr. P. U. Heitz
COMPREHENSIVE DIAGNOSTIC APPROACH USING SEVERAL DIAGNOSTIC TECHNIQUES
ELECTRON MICROSCOPY

• GENERALLY PROVIDES THE QUICKEST TAT- 24 HOURS FROM REQUEST
• SIGNIFICANT SAVINGS TO THE HEALTH CARE INDUSTRY, EVEN THOUGH IT MAY APPEAR TO BE MORE EXPENSIVE AT FIRST
• THE SAFEST IN TERMS OF OBTAINING THE CORRECT DIAGNOSIS

ELECTRON MICROSCOPY PITFALLS

• LACK OF KNOWLEDGE REGARDING SPECTRUM OF ULTRASTRUCTURAL FEATURES OF A GIVEN DIAGNOSIS
• INTERPRETING NORMAL STRUCTURES SURROUNDING A TUMOR AS PART OF THE TUMOR
• POOR FIXATION MASKING DIAGNOSTIC FINDINGS
• INTERPRETING FINDINGS IN A VACUUM

THE USE OF EM IN THE DIAGNOSIS OF SPINDLE CELL / EPITHELIOID TUMORS

• Establish specific diagnosis
• Rule out other types of tumors (LOOK-ALIKES) that may mimic a given diagnosis
• Clarify atypical (“aberrant”) IH results

Should formalin-fixed or paraffin embedded tumors be considered for EM EVALUATION?

• CERTAINLY, WITH SOME CAVEATS...
Formalin fixed is generally better than paraffin embedded tissue
PARAFFIN EMBEDDED TUMORS-
QUALITY FOR ELECTRON MICROCOPY DEPENDS ON A NUMBER OF VARIABLES
IN SOME CASES PRESERVATION IS EXTREMELY POOR AND EM IS OF NO VALUE
CONCLUSIONS

- EM can certainly play an important role in the differential diagnosis of spindle / epithelioid tumors and look-alikes
- The right diagnosis may be compromised if EM is not performed
- A comprehensive approach with use of a number of selective ancillary diagnostic techniques is undoubtedly the best way to approach unusual or difficult diagnostic situations

PROPER FIXATION OF SPECIMENS MAXIMIZES THE USEFULNESS OF ELECTRON MICROSCOPY
VALUE OF IMMUNOHISTOCHEMICAL STAIN IN THE EVALUATION OF SARCOMAS

- **CD 34**
  TYPICAL - dermatofibrosarcoma protuberans, endothelial tumors (a better marker than factor for these tumors?)
  PROBLEM - not tested extensively in other sarcomas

- **CD 38**
  TYPICAL - MFH
  ABERRANT - virtually any sarcoma can stain

- **Leu 7**
  TYPICAL - nerve sheath tumors
  ABERRANT - other sarcomas
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