60 Molecular Biology of Cartilage Neoplasia: Current Concepts and New Advances

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Chicago, IL 60603
This session will introduce the use and value of modern cytogenetic and molecular genetic approaches to help in the diagnosis cartilaginous tumors of bone. Topics to be covered range from osteochondroma through CMF and chondroblastoma to chondrosarcoma and its variants. Key to understanding will be the integration of the traditional demographic, imaging and histopathologic evaluation of these lesions with molecular approaches.

- Have an introduction to a selected subset of the more newly recognized cartilaginous tumors of bone.
- Be exposed to current molecular techniques used in the diagnosis of bone tumors.
- Identify and differentiate among a representative sample of the most common cartilaginous tumors of bone based on key demographic, clinical, imaging, histopathologic and molecular appearances.

FACULTY:

Gene Siegal MD, PhD
Practicing Pathologists
Surgical Pathology
Surgical Pathology (GI, GU, Etc.)
1.0 CME/CMLE Credit

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Molecular Biology of Cartilage Neoplasia: Current Concepts and New Advances

2011 ASCP Annual Meeting
WASPALM XXVI World Congress

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Gene P. Siegal, MD, PhD

Counterparts of fusion genes

Phenotype of the tumor

EWS
(Chromosome 22q11)

FLI-1
(Chromosome 11q24)

W5

Sm22q12)

ERG
(Chromosome 21q22)

ETV1
(Chromosome 7p22)

EIA-F
(Chromosome 17q21)

Ewing/PNET Phenotype of the tumor

Myxoid/round cell liposarcoma

Extraskeletal myxoid chondrosarcoma

Clear cell sarcoma

DSRCT
Primary ABC has been associated with:

- A chromosomal translocation t(16;17)(q22;p13).
- Fusion of the osteoblast cahedrin 11 (CDH11) promoter region on 16q22 juxtaposed to the ubiquitin-specific protease USP6 (Tre2) coding sequence on 17p13.
- Other 1st ABCs have CDH 11 or USP 6 rearrangements.
- Implication of a neoplastic basis for at least some ABC's.
**Fluorescence In Situ Hybridization (FISH)**

A cytogenetic technique used to detect and localize the presence or absence of specific DNA sequences on chromosomes.

**Alveolar rhabdomyosarcoma examined by FOXO1 (FKHR) gene break-apart probes.**

Note: Yellow signal in addition to green & red signals indicating a PAX3-FOX01 or PAX7-FOX01 gene fusion.

**Comparative Genomic Hybridization (CGH)**

A molecular cytogenetic technique to scan the genome for imbalances (duplications, deletions & copy number variants) i.e. copy number changes in the DNA content.

**Renal epithelial tumor (Clear Cell Carcinoma) Demonstrating -3p, +5q, trisomy 7, & monosomy 14**

**Spectral Karyotyping (SKY)**

A molecular cytogenetic technique to visualize all chromosomes pairs, in different colors, in order to identify structural chromosomal aberrations.

• Extraskeletal Myxoid Chondrosarcoma with a TAF2N-TEC gene fusion resulting from a complex (7;9;17) translocation.

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Chondroid Neoplasms

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<td>Bizarre Parosteal Osteochondromatous Proliferation</td>
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Chondroma
(Enchondroma, Periosteal Chondroma & Soft Tissue Chondroma)

No distinctive cytogenetic or molecular findings.

Chondroma Con’t.

Rare reports:
- Isochrome Ch 6 p10.
- Other Ch 6 alterations.
- t (12;15) (q13;p26).
- t (6;15) (q13;q11).
- Other alterations in arm Ch 12.
Chondroma Con’t.

- 12q 13-15 structural rearrangements.
- The HMGA2 (HMGIC) is the critical locus.
- By CGH gains have been seen on 13q21 and losses on Ch 19 and 22q.
- Ihh (critical in normal growth plate differentiation) appears to be absent in enchondromas while PTHrP signaling is active (but independent of Ihh).

Ollier’s Disease
(Multiple Enchondromatosis)

- Single report:
  - Del (1) (p11; p31.2).
  - Neither Ollier Disease nor Maffucci Syndrome is caused by activation mutation of PTHR1 gene.

Synovial Chondromatosis

- Ch 6 losses (? Site of collagen IX).
- ↑FGFR3 & ↑FGF9.
- FGF9 dependent on sonic hedgehog signaling.
- RAB23 – A negative regulator of Shh is located on 6p11.
Osteochondroma
- Majority spontaneous and solitary.
- Familiar form
  (Aut. dominant)
  (Hereditary multiple exostosis syndrome).

Osteochondroma
- EXT gene abnormalities in both forms.
- SKY suggests Ch 8 abnormalities and clonal changes in Ch 1.

Solitary (non-Hereditary) Osteochondroma
- High resolution 8q array CGH demonstrated homozygous deletion of EXT1 (as opposed to mutation).
- i.e. loss of both copies of EXT1 is required.
- These deletions were confined to the cartilage cap.
Hereditary Multiple Osteochondromas

- Mutation of either EXT1 (8q24) or EXT2 (11p11-p12) is involved.
- These act as tumor suppressor genes.
- Thus both copies need to be knocked out.
- Mutations in HMO patients results in truncated or non-functional proteins.
- The protein products of EXT 1/2 genes get “tied up” in the Golgi and can’t be expressed.


Deletion of 8q24

- Langer – Giedion Syndrome
  - Craniofacial dysmorphism
  - Mental Retardation
  - Multiple Osteochondromas

- Loss of functional copies of:
  - TRPS1 Gene
  - EXT1 Gene

Osteochondroma Mechanism

Osteochondroma con’t

- A recent paper has presented evidence that 0 of 11 sporadic osteochondromas showed biallelic inactivation of EXT genes while 5 out of 35 hereditary cases had a double hit suggesting alternative mechanisms to EXT genetic alterations are responsible for osteochondroma pathogenesis.  

- Primary cilia in osteochondromas are found randomly on cell surfaces. However, growth plate-like polarity was retained in some of these cells mimicking maturation in the normal growth plate. Thus, it is proposed that the cells found in the cartilaginous cap of osteochondromas are a mixture of normal (EXTwt/wt and EXT-/-) cells. 


Chondromyxoid Fibroma

- Complex rearrangements involving Ch 6. (Usually 6q13, 6q15, or 6q25)

- Unbalanced translocations between Ch 6 & 3. (note: Ch 3 [3p21] is the locus of PTHrP)

- Differences between conventional and juxtacortical forms.
Juxtacortical CMF

Chromosome break at Ch 6 between bands q12 & q13

CMF Cont’d

- Down regulation of N-cadherin, PTHLH (PTHrP), and PTHR1
- Up regulation (increased expression) of cyclin D1, p16, and Bcl 2.

Chondroblastoma

NO specific molecular or cytogenetic findings.
Chondroblastoma Con’t.

- Rare observations:
  - Ch 5 & 8 abnormalities
  - Ring Ch 4
  - Loss of collagen type II and replacement by type I.
  - Expression of active growth plate signaling molecules (FGF-2, FGFRI & 3, Ihh/PTHrP)
  - Recurrent breakpoints at 2q35, 3q21-23, and 18q21

BPOP (Nora’s Lesion)

- 1q32 break by FISH in 100%.
- 17q21 break by FISH in 80%.

BPOP (Nora’s Lesion) Cont’d

- 1 case of t(1;17) (q32;q21).
- 1 case with ring (Ch 12 by SKY)
- Note subungual exostoses all show a t(x;6) balance translocation.
Conventional Chondrosarcoma

Grading
- Evans system of three grades.
  - As one moves from low to high grade cytomorphology becomes pleomorphic, hyperchromatic and mitotically active.

Representative Case #1

- 59 y/o woman with a proximal humeral lesion

Conventional Radiography

- Intramedullary dense lesion of 7 cm
- Ring & arc type calcifications
- No periosteal reaction
- No cortical breakthrough
- Therefore “non-aggressive”
Histopathology
Representative Case # 2

- 63 y/o woman with a proximal humeral lesion
Conventional Radiography
The Radiologist labeled this lesion as "minimally aggressive"

Histopathology
Representative Case # 3

- 34 y/o woman with a radial lesion
Conventional Radiography

- Large lytic lesion
- Prominent lesional contents
- Permeative - multifocal scalloping
- No periosteal reaction or soft tissue mass

Histopathology
Representative Case # 4

- A 62 year old woman with a lesion of her left proximal fibula

Conventional Radiography

Histopathology
Representative Case #5

- A 23 year old young man with a lesion of his distal right femur

Conventional Radiography

- Epiphyseal centered lesion extending into metaphysis
- Sharp edges – Lesional content extends to subchondral plate
- No periosteal reaction
- Radiologist favors CMF

MRI

T1 T2
Purpose of the Study

- Inter-observer reliability in determining the grade of cartilaginous neoplasms

- Test platform: 46 consecutive cases of cartilaginous lesions in long bones that underwent open BX or curettage
3 Final Options

- Benign
- Low-grade Malignant
- High-grade Malignant

Results

- Pathologists: $\kappa=0.443$ ($p < 0.0001$)
- Radiologists: $\kappa=0.345$ ($p < 0.0001$)
- $\kappa =$ Kappa Coefficients

Conclusions

- Low reliability for grade determination
- Including low reliability in differentiating benign from malignant
Purpose of The Study

• Interobserved variability in histological diagnoses & grading

• Assess the diagnostic value of defined histologic parameters in differentiating enchondroma & grade I CS

Test Platform

• 16 Cases. Subsequently 20 enchondromas & 37 Grade I CS were examined.

• These later cases were fully worked up by multidisciplinary teams & 10 years follow-up obtained.
Results

- Histologic Assessment $\kappa = 0.78$
- Between enchondroma & Grade I CS
  $\kappa = 0.54$

### Parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Enchondroma</th>
<th>CS</th>
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<tbody>
<tr>
<td>Binucleated cells (&gt;2)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Nuclei Pleomorphism</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Condensed Nuclei</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Open Chromatin</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Mitosis</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Irregular Distribution of cells</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cellularity</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Encasement</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Host Bone Entrapment</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cortical Extension</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Additional Parameter

- Spontaneous pain (without pathologic fracture) was also found to be a significant discriminate $< p 0.05$
### UAB Nomenclature

- **Borderline lesion of indeterminant biological potential**

- **Cytogenetics & Molecular Genetics**

  The Future ???
Chondroma

No distinctive cytogenetic or molecular findings.

Genetics

- Peripheral & central CSs appear to arise through different aberrant genetic pathways.

Cytogenetics

- 6q 13-21 alterations associated with aggressiveness.
- Increased expression of PTHrP & Bcl-2. (when compared to chondromas)
Cytogenetics Con’t.

- Ch gains
  - 20q, 8q, 20p & 14q.
- Many Ch deletions
  - 13q (independent prognostic factor for metastasis).
  - Ch 1, 4, 5, 6, 7, 9, 10, 11, 12, 14, 18, 19, 20, 21, 22 & X also involved. (X, 6 & 18 most common)

Genetics Con’t

- Other signaling proteins:
  - PTHrP & bcl-2 (early event in peripheral lesions, late event in central).
  - Peroxisome proliferation activated receptor-gamma (PPARγ) in ~2/3 of cases.
  - JNK/ERK-AP-1/Runx2 pathway assoc. with histogenesis.

CXCR4

- Alterations in molecular elements derived from the CXC chemokine receptor 4 (CXCR4) cytokine system strongly correlate with neoplastic progression.
- Its ligand SDF-1 has been shown to be highly expressed in a variety of tissues where solid tumors are known to preferentially metastasize, such as lung, bone and lymph nodes.
CXCR4 Con’t

* It is already known to be involved in osteosarcoma metastasis.*


Hypothesis

* CXCR4 immunohistochemistry may be of value in separating low grade from high grade Chondrosarcoma.
Primary Metastasis

H&E

CXCR4

Punchline

• This technique did not allow for separation of enchondroma from Grade I chondrosarcoma.
Myxoid Chondrosarcoma

- t(9;22)(q22;q12) (5' EWS fused to TEC) (AKA: NOR1 & CHN)
- Resulting fusion protein
  - Transcription factor
  - Regulates mRNA splicing

Myxoid Chondrosarcoma Con't.

- 2 related translocations:
  - t(9;17)(q22;q11) in 15% of cases
    (fusion of TAF2N to TEC)
  - t(9;15)(q22;q21) (fusion of TCF12 to TEC)

Myxoid Chondrosarcoma Con't.

- cDNA microarray:
  - Not chondrocytic but rather primitive pluripotential mesenchymal cell phenotype.
  - ↑ neuremedin B (NMB)
  - Co-expression of EWS-TEC & SIX3 (A gene coding for a homotypic protein important for transcriptional activation).
Chondroblastic Osteosarcoma

- Aneuploidy common
- Approximately 2/3 of cases have chromosomal abnormalities (esp. gain of Ch 1 & loss of Ch 9,10,13 &17).
- CGH highlighted increased Ch complexity.

Chondroblastic Osteosarcoma Con’t.

- Rb gene alterations in 70% of cases.
- Other suppressor genes at 3q & 18q.
- Other OGS oncogenes include:
  SAS (36%),
  c-fos (60%)
  HER2/neu (40% mixed results).

Mesenchymal Chondrosarcoma

- No karyotypic abnormalities.
- der (13;21) (q10;q10)
- p53 expressions & mutation (~ 2/3 cases).
- sox9 (transcription factor important in differentiation).
Clear Cell Chondrosarcoma
- Allelic loss at 9p22 & 18q21.
- Methylation of p16 gene on Ch 9p.

Dedifferentiated Chondrosarcoma
- Almost no cases studied by cytogenetics or molecular genetics.
- Alterations of p53 (both overexpression and missense mutations).
- 1 case (MFH) near triploid karyotype with multiple Ch 7, deletion of 9p and der Ch 19.

Conclusions
- Ch 6 (col IX & Shh)
  - Chondroma
  - Synovial chondromatosis
  - CMF
  - Chondrosarcoma
- Osteochondroma
  - Ch 8 (EXT 1) / Ch 11 (EXT 2)
  - Ihh/PTHrP (also chondroblastoma & CS)
Conclusions Con’t

• Myxoid CS (not chondrocytic)
  TEC gene (Ch 9)
  t (9;15) – TEC – TCF12
  t (9;22) – TEC – EWS
  t (9;17) – TEC – TAF2N
• Ch 9 clear cell chondrosarcoma
• p53 dedifferentiated chondrosarcoma

Conclusions

• It remains difficult if not impossible to separate reproducibly enchondroma from Grade I CS by clinical, radiologic and/or histologic means.

• Focusing on “5 key” histologic criteria based on the EuroBoNet consortium may help narrow the difference.

Conclusions Con’t

• This still may result in a subset of lesions of “indeterminate biologic potential”.

• Advances in radiologic studies such as dynamic MR may further narrow this difference.

• Molecular genetic studies are the unproven future.
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