Sebaceous neoplasia: Diagnosis, immunohistochemical studies, molecular-genetics and relationships to Muir-Torre Syndrome

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Sebaceous neoplasia

- Histopathologic features of sebaceous gland proliferations
  - Sebaceous hyperplasia
  - Sebaceous adenoma
  - Sebaceous epithelioma
  - Sebaceous carcinoma

- Sebaceous gland neoplasia and the relationships to DNA mismatch repair

- Molecular genetic alterations of sebaceous carcinoma:
  - Differentially expressed miRNAs
  - Mutational signature in sebaceous carcinoma

Normal sebaceous glands
Normal sebaceous gland

Sebaceous hyperplasia
- Clinically small, flesh colored papules—typically with a central depression or umbilication on the face
- Benign overabundance of superficial sebaceous lobules surrounding a centrally dilated follicular structure normal-appearing sebaceous lobules
- Sebaceous lobules are increased in number and abut epidermis
- Sebocytes predominate over basaloid cells (only 1-2 layers)

Sebaceous hyperplasia

The spectrum of sebaceous neoplasia
- Sebaceous adenoma
- Sebaceous epithelioma/sebaceoma
- Sebaceous carcinoma

Not related to defects in mismatch repair
Sebaceous Adenoma

- Benign proliferation of sebocytes forming a yellow-tan papule or nodule
- Most commonly on the head and neck of older individuals
- Well circumscribed, lobulated nodule in the dermis
- Expansion of basaloid cells, but sebocytes predominate over basaloid cells (>50%)
- Established association with defects in mismatch repair
Sebaceous Adenoma

- Benign proliferation of sebocytes and basaloid cells forming a yellow-tan papule
- Most commonly on the head and neck
- Well circumscribed, lobulated nodule in the dermis
- Basaloid cells predominate over sebocytes
- Haphazard relationship between sebocytes and basaloid cells
- Established association with defects in mismatch repair

Sebaceous Epithelioma/Sebaceoma
Sebaceous Carcinoma is a rare but typically aggressive carcinoma that most commonly arises in an ocular location but can also arise in an extra-ocular location. Mainstay of initial therapy is surgical excision.

Clinical images courtesy of Dr. Bita Esmaeli.
Adipophilin is a sensitive and specific marker of sebaceous differentiation

Table 2: Adipophilin expression in sebaceous lesions and other cutaneous lesions, including lesions with clear cell histology

<table>
<thead>
<tr>
<th>Tumor cells staining (%)</th>
<th>Intensity of staining</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Negative (%) 1+ (%) 2+ (%) 3+ (%)</td>
</tr>
<tr>
<td>Sebaceous adenoma (n = 16)</td>
<td>0 (0) 0 (0) 0 (0) 16 (100)</td>
</tr>
<tr>
<td>Sebaceous carcinomas (n = 25)</td>
<td>0 (0) 0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Basal cell carcinoma (n = 10)</td>
<td>0 (0) 0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Squamous cell carcinoma (n = 12)</td>
<td>0 (0) 0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Adenocarcinoma, clear cell (n = 8)</td>
<td>0 (0) 0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Trichoblastomas (n = 8)</td>
<td>0 (0) 0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Xanthogranuloma (n = 10)</td>
<td>1 (10) 0 (0) 2 (20) 4 (40)</td>
</tr>
<tr>
<td>Xanthoma (n = 6)</td>
<td>0 (0) 0 (0) 2 (20) 4 (40)</td>
</tr>
<tr>
<td>Metastatic melanoma, melanoma (n = 4)</td>
<td>4 (100) 0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma, metastatic (n = 3)</td>
<td>4 (100) 0 (0) 0 (0) 0 (0)</td>
</tr>
</tbody>
</table>

Percentage 0, no labeling to 35% 1+, labeling in 36-75% of cells 2+, labeling in 76-99% and 3+, labeling in more than 99% of clear cells in the lesion.

Intensity 0, negative to weak. 1+, moderate to strong.
**Nuclear factor XIIIa is a sensitive and specific marker of sebaceous differentiation**

Nuclear factor XIIIa staining (clone AC-1A1 mouse monoclonal) is a highly sensitive marker of sebaceous differentiation in normal and neoplastic sebocytes.

- Squamous cell carcinoma (with clear cell changes) can occasionally exhibit weak to strong Factor XIIIa nuclear positivity.
- AC-1A1 clone specific—other Factor XIIIa antibodies (EP3372 Abcam or E980.1 Vector) do not stain sebaceous neoplasms.

**Sebaceous tumors and DNA mismatch repair**

- Sebaceous tumors are cutaneous markers for Muir-Torre Syndrome (MTS), an autosomal dominant cancer predisposition syndrome with cutaneous neoplasia (sebaceous and keratoacanthomas).
- MTS is a phenotypic variant of Hereditary Non-Polyposis Colon Cancer (HNPCC).
- Patients with MTS and HNPCC each at risk to develop:
  - Colorectal carcinoma.
  - Cancers of the endometrium, ovary, stomach, biliary tract, and genitourinary tract.

### Table 1. Nuclear staining for Factor XIIIa (AC-1A1) in sebaceous proliferations and squamous cell carcinoma (SCC)

<table>
<thead>
<tr>
<th></th>
<th>Strong</th>
<th>Focal</th>
<th>Weak</th>
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<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sebaceous hyperplasia</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sebaceous adenoma</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Sebaceous carcinoma</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>SCC</td>
<td>0</td>
<td>2</td>
<td>1</td>
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<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Adipophillin</td>
<td>94.0% (233/248)</td>
</tr>
<tr>
<td>Factor XIIIa AC-1A1</td>
<td>86% (49/57)</td>
</tr>
</tbody>
</table>
Muir-Torre: Germline defect in mismatch repair (MMR) genes leads to genomic instability

- MTS and HNPCC most commonly due to inherited defects in mismatch repair (MMR) genes: MLH1, MSH2, PMS2, MSH6.

Germline mutation in MMR gene

Acquired somatic mutation in other MMR gene: loss of function

Defective mismatch repair leads to accumulated genomic insults

Two complimentary methods test for defects in MMR:
- Immunohistochemistry assesses for integrity of MMR protein expression
- PCR interrogates integrity of microsatellite loci

Molecular studies confirm the microsatellite instability (MSI) phenotype

**Microsatellites**: repetitive DNA sequences (consisting of repeating units of 1-6 base pairs in length) scattered in the genome.

The length of a given microsatellite is highly variable, but each individual patient has microsatellites of a fixed length.
Sebaceous tumors from pre-selected patients show frequent DNA mismatch repair defects

- 16 patients with colorectal or endometrial carcinoma AND sebaceous skin tumors.
- Assessed skin and visceral tumors for microsatellite instability (MSI).
- ALL 16 patients exhibited MSI in their skin and visceral tumors.
- Among 13 patients tested, 9 patients (69%) had germline mutations in either MSH2 (8/9; 89%) or MLH1 (1/9; 11%)

- In patients with personal/family history of HNPCC/MTS associated visceral malignancy, testing for MMR defects is of high yield.
- In contrast to HNPCC (where MSH2 and MLH1 mutations are equally frequent), MTS is more often due to alterations of MSH2.

Sebaceous tumors from unselected patients show defects in DNA mismatch repair: Not related to germline defects...

- Identified 25 patients with sebaceous gland tumors (no history of visceral malignancy).
- Assessed sebaceous tumors for microsatellite instability (MSI).

What is the yield of MMR testing in unselected sebaceous tumors?

- ~60% unselected sebaceous neoplasms are MSI-High.
- ~50% patients with MMR-defective sebaceous tumors harbor germline defects.

Sebaceous tumors from unselected patients show defects in DNA mismatch repair: Not necessarily related to germline defects...

- Among 71 patients who had abnormal MMR IHC of ≥1 SN, 40 (56%) showed no germline mutation.
The challenge of sebaceous carcinoma:
Significant morbidity and frequent metastases

- Ocular Sebaceous Carcinomas are locally infiltrative tumors with frequent multifocal growth typically requiring aggressive surgery and can recur locally.

Surgical excision of sebaceous carcinoma can cause significant aesthetic morbidity

- Given their locally infiltrative pattern of invasion together with their frequent multifocal intraepithelial growth, sebaceous carcinomas typically require aggressive surgery.
Sebaceous carcinomas behave aggressively

- Early in their evolution ocular Sebaceous Carcinomas mimic benign processes, delaying diagnosis and definitive treatment.
- Metastases occur in ~10% of patients.
- Most common site is to parotid lymph node.

What are the molecular-genetic alterations in Sebaceous Carcinoma?

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Total sebaceous carcinomas tested</th>
<th>Number</th>
<th>Median age at diagnosis (y)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>20</td>
<td>63</td>
<td>49-88</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Ethnicity

- Caucasian: 15
- Hispanic: 3
- Asian: 1
- African-American: 1

Primary locally recurrent ocular sebaceous carcinomas: 18/20
Metastatic ocular sebaceous carcinomas: 5/3
Primary extra-ocular sebaceous carcinomas: 4/4

What are the molecular drivers of Sebaceous Carcinoma?

- Despite an aggressive clinical course, relatively little is known about the molecular pathogenesis of ocular adnexal Sebaceous Carcinoma.
- What are the molecular-genetic drivers of disease?
- Are there common mutations or frequently hijacked pathways typical of sebaceous carcinoma that might be leveraged in targeted therapies?

What are the molecular-genetic alterations in Sebaceous Carcinoma?

- Most common mutations:
  - TP53 in 14 OSC from 9 patients
  - RB1 in 9 OSC from 6 patients

- Coexisted in 5/16 patients

- Copy number changes rare in ocular sebaceous carcinoma

Ocular Sebaceous Carcinoma

- Among primary ocular adnexal sebaceous carcinomas:
  - Median = 3 mutation/lesion
  - Range = 0-16

- Most common mutations:
  - TP53 in 14 OSC from 9 patients
  - RB1 in 9 OSC from 6 patients

- Coexisted in 5/16 patients

- Copy number changes rare in ocular sebaceous carcinoma

Clinical images courtesy of Dr. Bita Esmaeli.
Mutations in *TP53* and *RB1* correlate with alterations in protein expression

Mutations predict activation of PI3-Kinase pathway in ocular Sebaceous Carcinomas

**PTEN** c.59G>A p.G20E

**TP53** c.743G>A p.R248Q
Mutations predict activation of PI3-Kinase pathway in ocular Sebaceous Carcinomas

Mutations in Sebaceous Carcinomas

- Together, these findings suggest PI3K-inhibitors as effective therapy.

Extra-ocular Sebaceous Carcinomas have mutations in DNA repair/chromatin remodeling and show high mutational burden

Extra-ocular Sebaceous Carcinomas

- Among extra-ocular Sebaceous Carcinomas (n=4)
  - Median= 23 mutations/lesion
  - Range= 3-29

- Only a subset of ~10 genes mutated in more than one lesion.

Somatic mutations in MMR genes in extra-ocular Sebaceous Carcinomas

- Three of four extra-ocular Sebaceous Carcinomas exhibited:
  - High mutational burden
  - Loss of Mismatch Repair (MMR) protein expression
  - Microsatellite (MSI) high phenotype by PCR
Somatic mutations in MMR genes in extra-ocular Sebaceous Carcinomas

Two patients with: Somatic MMR gene mutation, but NO detectable MMR gene mutation in the germline.

Model for Sebaceous Carcinomagenesis

- Somatic mutations in MMR genes (NO detectable MMR gene mutation in the germline) may occur in extra-ocular sebaceous carcinomas.
- Underscores the importance of tumor sequencing to exclude autosomal dominant cancer predisposition syndrome.
What is the composition and distribution of the tumor associated lymphoid infiltrate in sebaceous carcinoma?

Does sebaceous carcinoma express PD-L1?