Biomarkers in Ocular Melanoma
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Ocular Melanoma
Introduction

Ocular Melanoma

• Skin of eyelids
• Conjunctiva
• Uvea

Melanoma - Eyelids

• Rare eyelid tumor
• Basal cell, squamous cell and sebaceous carcinomas more frequent
• Associated with preexisting nevus or de novo
• Nevus > 0.5cm, irregular pigmentation and margins
Melanoma - Eyelids

Types
- Superficial spreading
  - Most common
  - Radial growth beyond invasive component
- Lentigo maligna melanoma
  - Face of elderly (pre-invasive form Lentigo Maligna)
- Nodular

Skin Melanoma

- Accounts for 75% skin-related deaths worldwide.
- Diagnosis is challenging due to large diversity of morphological patterns.
- Highest source of litigation in surgical pathology.

Melanoma - Diagnosis

Biomarkers

Patient

Identification of conditions

Predicts patient’s response to a treatment

Estimates progression or indolent behavior

Conventional Prognostic Markers

- Breslow thickness
- Clark level
- Growth phase (radial vs vertical)
- Tumor infiltrating lymphocytes (TILs) (density, type)
- Ulceration, present or absent
- Mitoses per mm²
- Status of sentinel lymph node (positive vs negative)
Histological staging

**Prognostic Features**

- **Depth of invasion (Breslow)**
  - Clark Level
  - 1
  - 2
  - 3
  - 4
  - 5

<table>
<thead>
<tr>
<th>10 YEAR Survival Rate</th>
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<tr>
<td>&gt;95%</td>
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<tr>
<td>&gt;75%</td>
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<td>&gt;55%</td>
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<td>&gt;35%</td>
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**Mitoses per mm²**

- **TILs**

**Immunohistochemical Markers**

- **S100**: Sensitive melanocytic marker but not specific. Excellent for desmoplastic melanoma.
  - Caviate: Usually negative or faintly positive in uveal melanoma.

- **HMB-45**: Helps differentiating benign nevi (decreased positivity with lesion depth/maturation) vs melanoma (consistent positivity in deeper part), not definitive in nevoid variants of melanomas.
**Immunohistochemical Markers**

Identification Biomarkers

- **MITF-1 & SOX-10:** Nuclear stains, best for lentiginous proliferations and pagetoid spread-difficult in-situ melanocytic lesions. Stain intraepithelial dendritic cells.
- **SOX-10:** High sensitivity for melanocytic differentiation. Used in desmoplastic melanoma along with S100 (recently reported in scar). **Caviate:** metastatic vs. breast, salivary gland carcinomas, neural crest tumors and clear cell sarcomas.
- **Ki-67 and pHH3:** Estimates proliferation rate (inflammatory cells). Co-staining with a melanocytic marker (HMB-45) may improve accuracy.

**Immunohistochemistry**

HMB45 (red) / Ki67 (brown)

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**Conjunctival Melanocytic Lesions**

- Recent growth of lesion in bulbar conjunctiva (15 year-old)

**Immunohistochemistry**

Melan A (red) / Ki67 (brown)

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**Clinical – Primary Acquire Melanosis (PAM)**

Fair skin, unilateral, flat conjunctival pigmentation
Biological diagnosis

Histologic diagnosis

Primary Acquired Melanosis (PAM)

Primary acquired melanosis (PAM) with atypia and Melanoma

No progression to melanoma

PAM with atypia

25-75% progression to Melanoma

25% mortality

PAM without atypia

PAM with atypia by bx.

Nodule developed – Melanoma by bx.

Clinical and Experimental Ophthalmology 2008; 36: 786–795


**Clinical Approach to Patient with Melanoma**

Biopsy Melanoma

Early Stage Late Stage

Microscopic Staging

Clinical staging

SLNBx

Imaging

LDH levels

Molecular staging

After tx

Targeted therapy

AJCC – staging and mutations

**Sentinel Lymph Node Bx (Skin & Conjunctiva)**

H&E

Melan A (immunohistochemistry)

**Molecular Classification**

- Highly heterogeneous disease
- Frequent driver mutations identified
- Regulate:
  - signal transduction pathways
  - developmental and transcriptional pathways
  - cell cycle

**Prognostic markers**

- Multimarkers assays
  - Single marker assays does not suffice in yielding enough prognostic or predictive information
  - Multimarkers give highly accurate information about prognosis or predictive response to therapy.
  - Prognostic multimarker signatures

- Epithelial to sarcomatoid morphology
- Inflammatory/immune pathways
- Angiogenesis
- Metastasis and invasion
Predictive markers

- Aim to predict patient response to treatment or combination of treatments.
- Could be very helpful in deciding personalized successful treatment.
- Very few tests are available clinically.

BRAF

- 50% of skin melanomas show mutations in BRAF gene.
- as an independent prognostic marker has shown conflicting data (found in benign nevi)
- vertical growth phase-melanomas = progression rather than genesis
- increased tumor thickness and ulceration
- Worse survival (5.7 months) for BRAF-mutant melanomas compared with BRAF-wild type cases (8.5 months)

BRAF

- 95% of the mutations found are at aa 600 leading to a constitutive MPAK/ERK pathway activation.
  - most commonly V600E (valine – glutamate)
  - sometimes V600K (valine – lysine)

Predictive markers

BRAF status to predict response to vemurafenib

- Vemurafenib improves PFS and OS in untreated melanomas carrying BRAF V600E mutation.
- Induced complete or partial tumor regression in 81% of BRAF + mutation patients.

BRAF

- Resistance due to upregulation of bypass pathways mediated by cancer Osaka thyroid kinase and the development of de novo NRAS or MEK mutations.

Predictive markers

Combined treatment response

- BRAF mutation can be either favorable (with cisplatin-vinblastine-temozolomide when associated with low O-6-methylguanine-DNA methyltransferase expression)
  - OR
- BRAF mutation can be detrimental (melphalan & actinomycin-D).
Predictive markers

**BRAF** - Testing

- BRAF mutations (V600E or V600K) can be detected from DNA samples from FFPE biopsy tissue through PCR testing.
- IHC for BRAF (V600E) shows excellent correlation with molecular-based analysis.
- Other point mutations of BRAF (V600K, V600Q, and V600R) melanomas do not stain positively with BRAF VE1 antibody.

**NRAS**

- 20% of melanomas show mutation of NRAS gene.
- NRAS as a prognostic indicator have shown mixed results
  - no difference in melanoma-specific survival compared with wild type tumors
- NRAS mutations frequently found in nevi and early melanomas
- Tumors with NRAS mutations are thicker (75% of NRAS mutated tumor were >1mm thick and had more than 1 mitosis/mm²)
- NRAS mutation is associated with poorer survival (this was not seen in low stage tumors, T2a or lower).

**KIT (c-kit)**

- Receptor tyrosine kinase that triggers downstream of different pathways (MAPK, PI3K, JNK and JAK/STAT) leading to cell growth, proliferation, migration and differentiation of melanocytes.
- Oncogenic potential of KIT in other tumors (GISTs, small-cell lung carcinomas and acute myeloid leukemia)
**KIT (c-kit)**

- KIT mutated in less than 5% of melanomas:
  - 30% of mucosal (conjunctival), 20% acral and 20% of melanomas arising in sun-damaged skin
- KIT in these specific scenarios behaves as an oncogene, providing a window to treat specific cases with KIT inhibitors

**Imatinib**
- An imatinib is a c-KIT inhibitor that is currently on phase II/III trials for patients with metastatic melanoma with c-KIT mutation.

**Predictive markers**

- **Predictors of immunotherapeutic agents**
  - No current biomarkers to predict success of immunotherapeutic agents (CTLA-4 (Ipilimumab) or PD-1/PD-L1 inhibitors (Atezolizumab)).

**PD-L1 (programmed death receptor ligand 1)**

- Negatively regulates T-cell function by binding to the PD-1 receptor on T cells = tumor evasion and proliferation
- Melanomas with aberrant expression of PD-L1 have poor prognosis
- Pembrolizumab and Nivolumab: human anti-PD-1 immune-checkpoint inhibitor antibody that blocks the interaction of PD-1 with PDL1 or another ligand PD-L2.

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**UVEAL MELANOMA**
Clinical Approach to Patient with Uveal Melanoma

- Clinical Dx
- Benign - Nevus
- Melanoma
- Radiation plaque
- Biopsy
- Enucleation
- Molecular Prognostic testing
- Confirmation Dx
- Metastatic screening (liver)

Most Common Malignant Intraocular Tumors

- Children
  - Retinoblastoma
- Adult
  - Metastases
  - Uveal Melanoma

Choroidal Melanoma

- Predisposing factors
  - melanosis oculi and Nevus of Ota
  - BAP1 associated tumor predisposition syndrome
  - 6 cases per million in USA (60-70 years of age)
  - 50% uveal melanoma patients will develop metastasis

Melanoma of the Uvea

- Location = symptoms specific to the location will develop and facilitate early or late diagnosis
- Ciliary body melanoma presents later because of location (hiding behind the iris/lens)
- Most frequent in whites and lightly pigmented individuals

Melanoma

- Iris = nonaggressive
- 3.3 – 16.6% are from iris (~10 years earlier dx than choroidal)

Clinical Diagnosis

- Standard of care
- Patients treated based on clinical/imaging features
Uveal Melanoma

- Metastasis 80% to liver (90% in liver prior to death)
- 1/3 of metastatic MM solely to the liver
- No difference in survival: local treatment with radiation plaque vs. enucleation (COMS prospective trial)

High Risk Prognostic Factors

Malignant Melanoma – Size, location

Callender Classification

Spindle A
Spindle B
Epithelioid
Mixed Melanoma

Vasculogenic Mimicry

Uveal Melanoma

Callender Classification

Spindle Melanoma
Epithelioid Melanoma
Mixed Melanoma

Five main genes are implicated in development and progression in UM:
- BAP1, EIF1AX, GNA11, GNAQ, and SF3B1

Genetic features associated with metastasis include:
- Monosomy 3 and gain of chromosome 8q
- BAP1, SF3B1, and EIF1AX mutations occur during UM tumor progression
- Mutually exclusive manner = different levels of metastatic risk.
**Molecular Heterogeneity**

FISH analysis on paraffin sections showed that heterogeneity of monosomy of chromosome 3 is a frequent phenomenon in uveal melanoma.


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**Molecular genetics in Uveal melanoma**

- **BAP1, SF3B1, and EIF1AX** occur later in tumor progression
- **Prognostically significant**

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**BAP1 – Epithelioid phenotype**

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**BAP1**

- Inactivating mutations in BAP1 are seen in 47% (27/57) uveal melanomas.
- Metastasis developed in the vast majority (26/27) of these tumors, many of which also showed monosomy of chromosome 3.
- These findings support the thesis that BAP1 is a classical tumor suppressor gene (Knudson’s two-hit model)
- One allele of BAP1 being lost via monosomy of chromosome 3 and the second allele being lost by inactivating BAP1 mutation/

**Molecular genetics in Uveal melanoma**

- BAP1 mutations recently reported to increase susceptibility for the development of uveal melanoma, cutaneous atypical and epithelioid melanocytic lesions, clear cell renal cell carcinoma, mesotheliomas and other tumors.

**BAP1 testing**

- Direct (Sanger) sequencing of the BAP1 gene using blood or salivary DNA from the individual(s) of interest (suspect of BAP1 related predisposition syndrome).
- BAP1 testing in tumors by RT-PCR
- BAP1 IHC in paraffin embedded tumor tissue (BAP1 loss of staining by IHC)
- Equivocal IHC results may undergo subsequent confirmatory sequencing.

**Gene expression profile analysis of primary uveal melanomas reveals two distinct tumor classes.**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Direction of Change in Class 2 Tumors</th>
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<tbody>
<tr>
<td>CDH1</td>
<td>E-cadherin</td>
<td>Up</td>
</tr>
<tr>
<td>ECM1</td>
<td>Extracellular matrix protein 1</td>
<td>Up</td>
</tr>
<tr>
<td>E1F1B</td>
<td>Eukaryotic translation initiation factor 1B</td>
<td>Down</td>
</tr>
<tr>
<td>FXR1</td>
<td>Fragile X mental retardation autosomal homolog 1</td>
<td>Down</td>
</tr>
<tr>
<td>HTR2B</td>
<td>5-hydroxytryptamine (serotonin) receptor 2B</td>
<td>Up</td>
</tr>
<tr>
<td>ID2</td>
<td>Inhibitor of DNA binding 2</td>
<td>Down</td>
</tr>
<tr>
<td>LMCD1</td>
<td>LIM and cysteine-rich domains 1</td>
<td>Down</td>
</tr>
<tr>
<td>LTA4H</td>
<td>Leukotriene A4 hydrolase</td>
<td>Down</td>
</tr>
<tr>
<td>MTUS1</td>
<td>Microtubule-associated tumor suppressor 1</td>
<td>Down</td>
</tr>
<tr>
<td>RAB31</td>
<td>RAB31, member RAS oncogene family</td>
<td>Up</td>
</tr>
<tr>
<td>ROBO1</td>
<td>Roundabout, axon guidance receptor 1</td>
<td>Down</td>
</tr>
<tr>
<td>SATB1</td>
<td>SATB homeobox 1</td>
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**GEP – Uveal Melanoma**

- Based upon the clinical outcomes from the prospective, 5-year multicenter Collaborative Ocular Oncology Group (COOG) study, the test reports Class 1A, Class 1B and Class 2 phenotype:
  - **Class 1A**: Very low risk, with a 2% chance of the eye cancer spreading over the next five years;
  - **Class 1B**: Low risk, with a 21% chance of metastasis over five years;
  - **Class 2**: High risk, with 72% odds of metastasis within five years.
Genes and GEP group

- **BAP1** mutations were associated with class 2 GEP ($P < .001$) and older patient age ($P = .007$).
- **EIF1AX** mutations were associated with class 1 GEP and the absence of ciliary body involvement ($P = .03$ for both).
- **SF3B1** mutations were associated with younger patient age ($P = .006$).
- **GNAQ** mutations were associated with the absence of ciliary body involvement ($P = .008$) and greater largest basal diameter ($P = .04$).
- **GNA11** mutations were not associated with any of the analyzed features.

PRAME

- Small percentage of Class 1 tumors result in metastatic disease.
- Cancer-testis antigen PRAME (preferentially expressed antigen in melanoma) = biomarker for increased metastatic risk in Class 1 tumors.
- Increased PRAME mRNA expression in Class 1 UM associated with transcriptional up-regulation of key genes involved in chromosome maintenance and stability (1q and 6p).

Molecular Mechanisms in UM

Feed-forward mechanism = progressively increasing PRAME expression and specific chromosomal gains mutually reinforce one another to promote Class 1 tumor progression.
- association with **SF3B1** mutations (mutually exclusive with **EIF1AX** mutations) → bifurcated pathway.
- This Class 1 metastatic pathway distinct from the bi-allelic loss of **BAP1** and acquisition of the Class 2 gene expression profile.
Requirement of Tissue for Molecular Testing

- Adequacy of sampling
  - GEP does not discriminate between normal, benign or malignant melanocytic lesion
  - Blood elements = Class 1
  - Some metastatic carcinomas = Class 2
- Confirmation of diagnosis
- FNABx before radioactive plaque
- Tissue retrieval at time of enucleation
- FFPE tissue acceptable

Cytology: Sampling prior to plaque

- Diagnosis and molecular prognosis of uveal melanoma

Examples of Current Trials

- MD Anderson Cancer Center
  - Elizabeth Grimm, MD; Sapna Patel, MD
  - Not stratifying based on any biomarkers
  - Usually test for GNAQ and GNA11 and alter as a positive trend at recurrence in these groups.
- Uveal melanoma does not have effective treatment for metastatic phase.
- GEP or other prognostic molecular testing shows no detrimental psychological effect in most patients.
- Early treatment of localized metastasis and possible enrollment in clinical trials.

Conclusions

- Oncogenes in melanoma are relevant for prognosis and therapeutic biomarkers.
- Immunohistochemistry as surrogate for mutations (BRAF, BAP1, PRAME).
- Noninvasive biomarkers needed (blood circulating tc).
- Immunoprofiling is a valid form of biomarker.
- Need for predicting response of immuno-based therapies.
- Epigenetic alteration are an expanding group of potential biomarkers.
- Special type melanomas have different tumorogenesis and prognostic biomarkers.
- Development of combination therapies appear to be important in melanoma.
THANK YOU