Prognostic and Molecular Basis of Melanoma

Martin C. Mihm Jr., M.D., F.A.C.P.
Harvard Medical School
Brigham and Women's Hospital
Dana Farber Cancer Center

Conflict of Interest

- Chairman Scientific Advisory Board – Caliber I.D. Inc.
- Member Scientific Advisory Board – MELA Sciences Inc.

Malignant melanoma

- In situ
- Invasive
Malignant Melanoma In Situ

Invasive Melanoma

• Nontumorigenic:
  Radial Growth Phase
• Tumorigenic:
  Vertical Growth Phase

Radial Growth Phase
Radial Growth Phase

• In Situ: confined above basement membrane
• Microinvasive: biologically indolent and common

[none of 161 patients showed metastases at a mean Follow-up of 13.7 years]


Radial Growth Phase
Characteristics

• Cells present individually or in small nests
• Dermal nests are no larger than epidermal nests
• Dermal mitoses are absent
• Dermal component usually confined to papillary dermis
• Papillary dermis is usually no expanded
• No single group of cells is substantially larger than any other group
• Dermal and epidermal cells are cytologically similar
Vertical Growth Phase
Vertical Growth Phase

- Dermal cells present in one or more expansile nodules
- Dermal nests are larger than nests in epidermis
- **Dermal mitoses**
- Dermal component often extends into reticular dermis
- Papillary Dermis is often expanded
- Dermal and epidermal cells are cytologically different

Four Common Forms of Malignant Melanoma

Three with a Radial Growth Phase [RGP]
- Superficial Spreading Melanoma  70%
- Acral Lentiginous Melanoma     8%
- Lentigo Maligna Melanoma       5%

One with only Vertical Growth Phase [VGP]
- Nodular Melanoma               15%
Malignant Melanoma: Acral Lentiginous Type
Malignant Melanoma: Lentigo Maligna Type
Malignant Melanoma: Nodular Type?

PROGNOSTIC FACTORS

Levels of Invasion (Clark’s levels)
- Level I: confined to the epidermis and appendages
- Level II: papillary dermis
- Level III: filling the papillary dermis, impinging the reticular dermis
- Level IV: broad front in the reticular dermis
- Level V: subcutaneous fat
Tumor Thickness
(Breslow’s thickness)

- Single most important factor in predicting survival for stage I patients
- Primary determinant of T staging:
  - T1: up to 1.0 mm
  - T2: 1.1-2.0 mm
  - T3: 2.1-4.0 mm
  - T4: >4.0 mm
FACTORS ASSOCIATED WITH AGGRESSION IN THIN MELANOMAS

- Early vertical growth phase
- Ulceration
- Level IV
- Mitosis
- Extensive regression

THESE FACTORS SHOULD LEAD TO CONSIDERATION OF SENTINEL LYMPH NODE BIOPSY.
Brisk Lymphocytic Response

TILs are present throughout the substance of the vertical growth phase or present infiltrating Across the entire base of the vertical growth phase.

Clark WH et al. JNCL. 81:1983,1988
Non-Brisk Lymphocytic Response

Tils are present in one or more foci of the vertical growth phase

Clark WH et al. JNCI.81:1983,1988
The lymphocytes to be of prognostic import must infiltrate and disrupt the tumor cells of VGP

Microscopic Satellite

- Nest(s) of malignant melanocytes,
- >0.05 mm. in diameter in reticular dermis,
- Present in panniculus or vessels beneath the tumor mass but separated from it by normal tissue in the section in which the Breslow measurement made

Prognostic Factors: Microscopic Satellite
Ulceration is defined as microscopic interruption of the surface epithelium by tumor.

Reference:

Ulceration

• Full thickness interruption of the epidermis by the tumor without history of prior surgery or trauma at the site, accompanied by reactive changes such as inflammation and fibrin deposition.

Prognostic Factors: Ulceration
**Regression**

- Area of epidermis without recognizable tumor flanked by obvious melanoma. Deep to the tumor free epidermis, the papillary dermis is also free of tumor and usually widened because of delicate fibrous tissue with increased vascularity and scattered melanophages.
Dermal Mitoses

- One of the most significant prognostic factors in the latest AJCC staging system.
- Count dermal mitoses only.
- Number of mitoses per mm².
- 1mm² = approx. 4 to 5 HPF’s (40X).
Prognostic Factors: Dermal Mitoses (Hot Spot)
A Prognostic Tree for 10-Year Metastasis in AJCC Stage 1 Melanoma (Breslow thickness < 1 mm)

Outline

- Cell cycle markers
- Molecular markers of prognosis/progression

Cell-cycle Markers/Regulators

- Direct measurement:
  - PCNA: unreliable with antigen retrieval
  - Ki-67(MIB-1): preferred method using MIB-1 clone active in paraffin tissues
  - Cyclins: IHC studies; Cyclins B and D of interest
  - Cyclin regulators: p21\textsuperscript{WAF1}; p27\textsuperscript{Kip1}; p34\textsuperscript{cdc2}
- Present in significant higher rates in VGP vs RGP
- Achieved independent prediction of outcome in some studies
- May differentiate melanoma from benign atypical nevi
Active cell proliferation by MIB-1 staining

Outline

• Cell cycle markers
  • Molecular markers of progression/prognosis

Oncogenes: BRAF and Melanoma

• Ras mutations identified in 9-15% of melanomas.
• BRAF somatic missense mutations identified in 60-66% of melanomas.
• 80% BRAF mutations involve a single substitution of glutamate for valine (V600E).
• V600E mutation (insertion of a negatively charged amino acid) mimics a phosphorylation on the regulatory domain of BRAF.
• Present in intermittently sun exposed skin (Bastian et al., 2003, 2006)
MAP Kinase Signaling Pathway

- Upon binding of a ligand, RTK dimerizes and triggers autophosphorylation.
- RTK binds to adaptor protein (GRB2), which recruits SOS.
- Binding of SOS to RAS results in GTP-bound RAS conformation initiation of phosphorylation cascade.

Increased ERK Activity in Melanoma Invasion

Integrins

heterodimeric cell surface receptors
- αvβ3 - confers invasive properties on melanoma cells transfected into nude mouse, expression correlates with VGP
- α4β1 - confers metastatic properties to human melanoma cells transfected into nude mouse
αvβ3 Integrin Expression in Melanoma

β3 Integrin Over-expressed in a Thick Melanoma
β3 Integrin Not Expressed in a Thin Melanoma

β3 Integrin Expression and Survival

UPenn Group

Prognostic Significance of Integrins in comparison to established pathologic factors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariate P value</th>
<th>Multivariate P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β3 integrin +/-</td>
<td>&lt;0.0001</td>
<td>0.002</td>
</tr>
<tr>
<td>No. of LN+</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Clark's level</td>
<td>0.031</td>
<td>0.011</td>
</tr>
<tr>
<td>β1 integrin +/-</td>
<td>0.004</td>
<td>0.05</td>
</tr>
<tr>
<td>Mitosis/mm²</td>
<td>0.05</td>
<td>0.23</td>
</tr>
<tr>
<td>Thickness</td>
<td>0.04</td>
<td>0.24</td>
</tr>
</tbody>
</table>
Cell surface adhesion molecules and their expression in melanoma

Bogenrieder et al, 2003

Cadherin Molecules – affect location of melanoma in the skin

• E-cadherin is expressed on keratinocytes and melanocytes, apparently the major adhesion molecule between epidermis and keratinocytes. Loss is associated with upregulation of MCAM and αvβ3 in the RGP, freeing melanocytes from their keratinocyte-linked environment.
• N-cadherin is expressed on fibroblasts and endothelial cells and melanoma cells in the dermis allowing for N-cadherin mediated adhesion as well as connexion-mediated gap junctions with fibroblasts and endothelial cells and other melanoma cells.
• E-cadherin is intimately associated with the cytoskeleton through the catenins

Bogenrieder et al, 2003

Matrix Metalloproteinases

• Mediate degradation of extracellular matrix in process of invasion
• MMP-2 correlated with increased malignancy in melanoma mouse model (Hofmann et al., 1999)
• MMP-9 has been shown to be highly expressed in human melanoma cells in focal fashion implicating it in focal clonal selection and expansion (Bodey et al, 2001)
**Nature 2002**
Davies et al.

BRAF mutations in 7% of solid tumors
60% of melanoma


**Natural history of BRAF mutation**

Selective BRAF & MEK inhibitors in BRAF mutant melanoma
PLX4032: phase I study design

Open-label, sequential dose escalation study

Primary objectives:
• Safety and PK of PLX4032 in patients with solid tumors
  - 49 of 55 phase I patients had metastatic melanoma
• Assess response rate and progression-free survival

Secondary objectives:
• Measure impact of PLX4032 on:
  - 18FDG uptake by PET
  - pMEK, pERK & Ki67 in paired tumor biopsy samples

Eligibility standard for phase I trials; no requirement for BRAF^V600E
  - increasing percentage of patients prospectively genotyped

Drug-related toxicities seen in >10% of patients in extension cohort at MTD (960 mg BID)

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>All Grades</th>
<th>Grade ≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>68 %</td>
<td>3 %</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>48 %</td>
<td>3 %</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>42 %</td>
<td>3 %</td>
</tr>
<tr>
<td>Fatigue</td>
<td>32 %</td>
<td>7 %</td>
</tr>
<tr>
<td>Cutaneous SCC (keratoacanthoma)</td>
<td>23 %</td>
<td>23 %</td>
</tr>
<tr>
<td>Pruritis</td>
<td>23 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Palmar-plantar dysesthesia</td>
<td>23 %</td>
<td>3 %</td>
</tr>
<tr>
<td>Nausea</td>
<td>19 %</td>
<td>3 %</td>
</tr>
<tr>
<td>Alopecia</td>
<td>16 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>13 %</td>
<td>3 %</td>
</tr>
</tbody>
</table>
Partial response in lung metastases

Pre-treatment 2 months

Partial response in bowel metastasis

Pre-treatment 2 months

Partial response in liver metastases

Pre-treatment 2 months 4 months
Strategy for building on single-agent oncogene directed therapy

• Establish molecular & clinical consequences of individual signaling inhibitors

• Develop combination regimens based on concomitant activation of oncogenic pathways

• Develop sequential treatment strategies based on the observed mechanisms of resistance

Immunotherapy

• Nature has developed natural checkpoints in the immune response to prevent us from having total autoimmunity

• There are 2 regulatory molecules that inhibit the immune response:
  – CTLA4 antigen that inhibits immune reactions
  – PDLR on tumor cells that results in inactivation or destruction of T cells that present its ligand

• Current immunotherapy includes an anti-CTLA4 antibody and an inhibitor of the PDLR that result in enhanced immunity and better survival in melanoma

Acknowledgments

• Klaus Busam MD
• Kerry Crotty MD
• Harry Kozakewich MD
• Adriano Piris MD
• Nicolas Prieto MD
• Victor Prieto MD
• Cecilia Lezcano MD
• Richard Scolyer MD