Molecular Epidemiology of BRAF and NRAS Mutations in Melanomas

Nancy E. Thomas, MD PhD
Associate Professor
University of North Carolina
at Chapel Hill
Questions

Do childhood and adult sun exposure increase melanoma risk?

Do common NER polymorphisms increase melanoma risk?

Are melanoma pathways (denoted by mutational status) differentially associated with sun exposure and moles?

Is there a mechanism by which BRAF mutations could arise related to sun exposure?
Genes, Environment, and Melanoma Study

“GEM”
GEM Study Design

Multiple Primary "CASES"
- n = 1238

Single Primary "CONTROLS"
- n = 2485

Sun exposure and phenotypic data

Inherited genetic markers
- DNA repair polymorphisms

Somatic genetic markers
- (tumor blocks)
  - BRAF, RAS
Interpretation of Results

Risk of second or higher order melanoma among persons with a first diagnosis of melanoma

approximates

Risk of first primary melanoma among persons who were previously unaffected

Begg. Int J Epidemiol, 2006
## Sunlight Exposure in GEM

<table>
<thead>
<tr>
<th>Ecologic level</th>
<th>Residence history linked to latitude, zenith angle, ozone column, surface elevation, cloud cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual level</td>
<td>Recreational, occupational, vacations, sunburns</td>
</tr>
</tbody>
</table>
### Strong Melanoma Risk Factors

<table>
<thead>
<tr>
<th>Ecologic level</th>
<th>Childhood sun exposure by residence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR ~ 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Individual level</th>
<th>Lifetime beach activities &amp; holidays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR ~ 1.5</td>
</tr>
</tbody>
</table>

Kricker et al. Cancer Causes Control, 2006
DNA repair genes

Nucleotide excision repair gene polymorphisms

XPD, HR23B, XPG, XPC, XPF, ERCC6
DNA repair genes

\begin{align*}
XPD 312 & \quad \text{OR} = 1.5 \ (1.2-1.9), \ P = 0.004 \\
XPD 751 & \quad \text{OR} = 1.4 \ (1.1-1.7), \ P = 0.004
\end{align*}

Strongest for diagnosis before age 30.

Number of \( XPD \ 312 + 751 \) haplotypes:
\begin{align*}
\text{trend } P &= 0.002
\end{align*}

Millikan et al. Carcinogenesis 2006
DNA repair genes

Increased risk with increasing number of variant alleles for all NER genes combined:

\[ \text{trend } P = 0.02 \]
GEM Results

Somatic genetic markers

(tumor blocks)

BRAF, RAS
Nested GEM Study

214 cases in North Carolina, 2000
NC Cases (N=214)

Mean 51.8 years; 55% male

55.5% thin (< 0.75 mm)

79.7% SSM, 4.5% NM, 10.4% LMM, 5.5% other
Mutually Exclusive

BRAF+
NRAS-

BRAF-
NRAS+

BRAF-
NRAS-
(wildtype)
MAPK Kinase Pathway Activation

Growth Factors → C-KIT → RAS → BRAF → MEK → ERK → TF → Cell Proliferation & Survival
Prevalence

**Wildtype**
93 (43%)

**BRAF Mutant**
92 (43%)

**NRAS Mutant**
29 (14%)
NRAS+ Decade older than BRAF+

P < 0.0001

BRAF+: 47 yrs
NRAS+: 62 yrs

Thomas et al., CEBP 2007
Anatomic Site

BRAF+

NRAS+

BRAF-

NRAS-

(wildtype)

Similar Evidence in SEER

Age-density distribution
2000-2004
(n=48,673)
Lachiewicz et al.
JID 2007

Proportion of cases

Age at diagnosis

Trunk

Face, ears

Proportion of cases

Age at diagnosis

Lachiewicz et al. JID 2007
North Carolina GEM Cases Tumor characteristics

<table>
<thead>
<tr>
<th>BRAF +</th>
<th>• SSM, NM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Low solar elastosis</td>
</tr>
<tr>
<td>NRAS +</td>
<td>• SSM, NM</td>
</tr>
<tr>
<td>BRAF – RAS –</td>
<td>• LMM</td>
</tr>
<tr>
<td></td>
<td>• High solar elastosis</td>
</tr>
</tbody>
</table>
Histologic Evidence of Solar Elastosis

Homogenization of the superficial dermis
Erythemal UV Irradiance

Erythemal UV (250-400) KJ/m\(^2\)/yr 1979-2000 avg.
## Ambient Erythematous UV Exposure

<table>
<thead>
<tr>
<th>Ambient Annual UV</th>
<th>BRAF+ vs WT Age-adj OR (95% CI)</th>
<th>NRAS+ vs WT Age-adj OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lifetime</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low UV</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>High UV</td>
<td><strong>2.0 (1.0-4.0)</strong></td>
<td><strong>1.1 (0.4-2.7)</strong></td>
</tr>
<tr>
<td><strong>Early life</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low UV</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>High UV</td>
<td><strong>2.6 (1.2-5.3)</strong></td>
<td><strong>0.9 (0.4-2.2)</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High UV irradiance</th>
<th>(BRAF^+) vs Wt Age-adj OR (95% CI)</th>
<th>(NRAS^+) vs WT Age-adj OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth year</td>
<td>2.0 (1.0-4.1)</td>
<td>0.9 (0.4-2.2)</td>
</tr>
<tr>
<td>Age 10</td>
<td>1.9 (1.0-3.9)</td>
<td>0.8 (0.3-1.9)</td>
</tr>
<tr>
<td>Age 20</td>
<td>2.7 (1.3-5.7)</td>
<td>0.8 (0.3-1.9)</td>
</tr>
<tr>
<td>Age 30</td>
<td>1.0 (0.5-1.9)</td>
<td>0.7 (0.3-1.8)</td>
</tr>
<tr>
<td>Age 40</td>
<td>1.4 (0.6-3.3)</td>
<td>1.3 (0.5-3.4)</td>
</tr>
<tr>
<td>Age 50</td>
<td>1.2 (0.4-3.8)</td>
<td>2.5 (0.7-8.5)</td>
</tr>
<tr>
<td>Age 60</td>
<td>1.1 (0.2-7.0)</td>
<td>2.0 (0.4-9.8)</td>
</tr>
</tbody>
</table>
BRAF and NRAS Mutations in Moles

- About 70% of moles have BRAF mutations
- Some moles have NRAS mutations
- Great majority of moles do not progress to melanoma

Pollock Nat Genet 2002; Kumar JID 2003; Yazdi JID 2003
# Associations with Moles

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BRAF&lt;sup&gt;+&lt;/sup&gt; vs WT Age-adj OR (95% CI)</th>
<th>NRAS&lt;sup&gt;+&lt;/sup&gt; vs WT Age-adj OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Back mole counts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5-14</td>
<td>2.4 (1.1-5.5)</td>
<td>1.2 (0.4-3.7)</td>
</tr>
<tr>
<td>&gt; 14</td>
<td>3.2 (1.4-7.0)</td>
<td>1.7 (0.6-4.8)</td>
</tr>
<tr>
<td>$P_{trend}$</td>
<td>0.006</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Mole density diagrams</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Low</td>
<td>2.3 (1.0-5.2)</td>
<td>2.7 (0.8-8.6)</td>
</tr>
<tr>
<td>Medium to high</td>
<td>3.8 (1.4-10.4)</td>
<td>3.3 (0.7-14.9)</td>
</tr>
<tr>
<td>$P_{trend}$</td>
<td>0.009</td>
<td>0.10</td>
</tr>
</tbody>
</table>
### Multivariate Model

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>$BRAF^+$ vs Wt</th>
<th>$NRAS^+$ vs Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at diagnosis (per 10 yrs)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8 (0.7-1.0)</td>
<td>1.4 (1.1-1.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Back mole counts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5-14</td>
<td>2.8 (1.2-6.4)</td>
<td>1.1 (0.4-3.3)</td>
</tr>
<tr>
<td>&gt; 14</td>
<td>3.4 (1.5-7.8)</td>
<td>1.9 (0.6-5.5)</td>
</tr>
<tr>
<td>$P_{trend}$</td>
<td>0.004</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Early life UV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low UV</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>High UV</td>
<td>2.6 (1.2-5.6)</td>
<td>0.9 (0.4-2.2)</td>
</tr>
</tbody>
</table>
Are Moles Causal Intermediates for Some $BRAF^+$ Melanomas?

- Mole-prone individuals are more likely to have $BRAF^+$ melanoma

- Presence of a contiguous mole was associated with $BRAF^+$ melanomas
Melanoma Models

Mole Prone
Early life
BRAF+
Later life?
NRAS+
Mole Resistant
Habitual Sun?
NRAS-BRAF-

↑ AGE
Tandem BRAF Mutations

10% of melanomas; rare in other BRAF-mutant tumors

Tissue-specific UV exposure?

Proposed mechanism:
Nearby potential pyrimidine dimer sites
Specialized DNA polymerases

**BRAF Mutations in Melanomas**

Wild-type:

3' **CGATGAC**ACTTTAGA
5' **GCTACAG**TGAAATCT

* Di-pyrimidines, potential sites for photoproduct formation

* * *

* * *

* * *

Mutagenic bypass of UVB-induced DNA lesions?

3' **CGATGAC**TCTTTAGA
5' **GCTACAG**AGAAATCT

t1799a Mutant

3' **CGATGAC**TTTTTTAGA
5' **GCTACAG**AAAAATCT

tg1799aa Tandem Mutant
Inaccurate Polymerization?

Thomas NE, Berwick M, Cordeiro-Stone M, JID 2006
Childhood & adult sun exposure increase melanoma risk

Common NER polymorphisms increase melanoma risk
  > OR with high waterside sun exposure

Melanomas pathways are differentially associated with sun exposure, modified by nevus propensity

BRAF mutations could arise from a mechanism involving nearby potential pyrimidine dimer sites, specialized DNA polymerases, and powerful selection
Future Plans

7 GEM sites participating in somatic tumor BRAF NRAS analysis
~1000 cases for analysis of risk and outcome

Relationship of XPD polymorphisms with NRAS and BRAF somatic mutations is being examined
Collaborators

**UNC-GEM Melanoma Group**
Robert Millikan  
Kathleen Conway  
Pam Groben  
David Olilla  
Bill Kaufmann  
Marila Cordeiro-Stone  
Norman Sharpless  
Sharon N. Edmiston  
Audrey Alexander  
Honglin Hao  
Anne Lachiewicz  
Dianne Mattingly  
Jessica Tse  
Janiel Shields

**GEM Melanoma Group**
Marianne Berwick  
Colin Begg  
Klaus Busam  
Bruce Armstrong

**NCAR - Atmospheric Chemistry Division**
Julia Lee-Taylor

**Univ New Mexico**
Charles Wiggins

This research was supported, in part, by:
- NCI
- NIEHS
- Center for Environmental Health & Susceptibility
- Lineberger Comprehensive Cancer Center
- Dermatology Foundation