Linking the pigmentary response to DNA repair in melanocytes

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Skin Cancer

- >50% of all new cancers (U.S.)
- Keratinocyte malignancies
  - most common (> 1,000,000 per yr in US)
    - basal cell carcinoma (BCC)
    - squamous cell carcinoma (SCC)
- Malignant melanoma
  - most deadly
    - Account for ~¾ of the 10,000 annual US deaths from skin cancer
  - Increasing prevalence across age groups (even pediatrics)
    - Highest incidence in males > 50 yrs
    - #1 cause of cancer deaths among women ages 20-25 yrs.

*American Cancer Society’s Facts and Figures*
Melanoma

- Cancer of melanocytes.
- Incidence rising for several decades.
  - ~ 60,000 new cases a year
  - ~ 8,500 deaths
- Top-ten cancer, both sexes
- Essentially insensitive to chemotherapy or radiation therapy.
- New therapeutic advances

SEER data, National Cancer Institute, and data from the American Cancer Institute
**Recent advances in Melanoma therapy**

**BRAF inhibition**

- ~50% of melanomas carry an activating mutation of the *BRAF*
  - serine–threonine protein kinase in the MAP kinase cascade

- Treatment of BRAF-mutant melanoma with PLX4032 (BRAF inhibitor) resulted in tumor regression in the majority of patients
  - Overall survival 10.1 months (vs. 6.4 months)

- **BUT…** the majority of patients on early trials of these drugs develop secondary resistance and subsequent disease progression

**Immune modulation**

- Improved survival (by ~4 months) in patients with advanced melanomas receiving ipilimumab + gp100vaccine
  - Ipilimumab mAb that binds to CTLA-4 on cytotoxic T cells to sustain anti-cancer immunity.

- gp100 peptide vaccine + IL-2 led to better clinical responses and overall survival in metastatic melanoma patients
  - 16% vs. 6% overall clinical response
  - Progression-free survival (3.9 vs. 1.6 mo)
  - Better overall survival (17.8 vs. 11.1 mo)

Flaherty et al., NEJM, 2010  
British Journal of Cancer, 2011  
Hodi et al., NEJM, 2010  
Schwartzentruber et al., NEJM, 2011
Melanoma Risk Factors

• Fair skin, freckling and light hair
  – *Inability to tan effectively*

• Defective Nucleotide Excision Repair
  • *Xeroderma pigmentosum (XP)*

• Moles
  – *Dysplastic nevus syndrome*

• UV radiation
  – *Blistering sunburns*

• Immune suppression

• Family or personal history

• Age

• Inherited cancer predisposition
  – *Familial melanoma (p16)*
Skin pigmentation phenotype – a major determinant of melanoma risk
Melanocyte

- Neural crest derived cell
- Exclusive pigment producing cell in the skin
- Mainly in the skin
  - Leptomeninges (medulla oblongata)
  - Eye (retina)
  - Inner ear (ionic gradient)
- Precursor cell for malignant melanoma

*Primary melanocytes in culture*
The Skin
“Epidermal Melanin Unit”

**EPIDERMIS**
- Keratinocytes
- Stratum corneum
- Stratum lucidum
- Stratum granulosum
- Stratum spinosum
- Stratum basale

**DERMIS**
- Melanocytes

**Note:** The diagram illustrates the layers and cells in the epidermis and dermis, highlighting the relationships and locations of keratinocytes and melanocytes.
## Skin Pigmentation: Fitzpatrick Scale

<table>
<thead>
<tr>
<th>Skin Phototype</th>
<th>Constitutive Skin Color</th>
<th>MED (mJ/cm² UVB)</th>
<th>UVB Sensitivity</th>
<th>Tanning/Burning History</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Ivory/pale white</td>
<td>15-30</td>
<td>+++</td>
<td>Burns easily and strongly, never tans</td>
</tr>
<tr>
<td>II</td>
<td>Very white</td>
<td>25-40</td>
<td>+++/++++</td>
<td>Burns easily, tans minimally with difficulty</td>
</tr>
<tr>
<td>III</td>
<td>White</td>
<td>30-50</td>
<td>+++</td>
<td>Burns moderately, tans somewhat</td>
</tr>
<tr>
<td>IV</td>
<td>Light brown, beige, olive</td>
<td>40-60</td>
<td>++</td>
<td>Burns minimally, tans moderately</td>
</tr>
<tr>
<td>V</td>
<td>Moderate brown</td>
<td>60-90</td>
<td>+</td>
<td>Rarely burns, tans well</td>
</tr>
<tr>
<td>VI</td>
<td>Dark brown/black</td>
<td>90-150</td>
<td>+/-</td>
<td>Never burns, tans profusely</td>
</tr>
</tbody>
</table>

Melanin biosynthesis

Tyrosine → DOPA → DOPAquinone → LeucoDOPAchrome → CysteinylDOPA → 1,4-benzothiazinylalanine → Eumelanin (brown/black melanin)
- very effective UV blocking pigment

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Eumelanin is a Terrific Natural Sunscreen!

- Skin cancer incidence correlates with pigmentation
  - 20-30% of all neoplasms in Caucasians.
  - 2-4% of all neoplasms in persons of East Asian inheritance
  - 1-2% of all neoplasms in persons of African or Asian-Indian descent
- The dose of UVR required to produce sunburn is up to 30 times greater in people of color than in Caucasians.

<table>
<thead>
<tr>
<th>% of UV Radiation that gets through the epidermis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darkly Pigmented Skin</td>
</tr>
<tr>
<td>UV-A</td>
</tr>
<tr>
<td>UV-B</td>
</tr>
</tbody>
</table>

US Melanoma Incidence by Race, 2004-08

Incidence rate (per 10^5/yr)

- All Races
- White
- Black
- Asian/Pacific Islander
- American Indian/Alaska Native
- Hispanic

Legend:
- Men
- Women
Skin complexion is multi-genic

- **MATP**
  - Membrane-associated transport protein
  - Sugar transporter in melanosome membrane
- **MC1R**
  - Melanocortin 1 receptor, Gs-coupled protein
  - Binds MSH on surface of melanocytes
- **SLC24A5**
  - Solute carrier family 24, member 5
  - Cation exchanger in melanosomes, cysteine transport
- **ASIP**
  - Agouti signaling protein
  - MC1R antagonist
- **P**
  - Pink-eyed dilution protein
- **Tyr**
  - Tyrosinase
  - Oculocutaneous albinism type 1
- **DCT**
  - Dopachrome tautomerase
  - Oculocutaneous albinism type 4

Correlates with fair skin, red hair, freckling, inability to tan and melanoma risk in humans

- C. de Torre et al., *Melanoma Res* 20, 342 (2010).
Mechanism of Adaptive Pigmentation

Cui, et. al., Cell, 2007
MC1R defects correlate with:

- Fair-skin
- Inability to tan
- Melanoma risk

**MC1R Function**

- Good cAMP signaling
- Loss of function

**Eumelanin → melanin found in skin ← Pheomelanin**

**Skin phototype (complexion); Fitzpatrick scale**

VI  V  IV  III  II  I

- Never Burns
- Tans easily
- Can’t tan
- UV sensitive

**Melanoma Risk**

**MC1R mutations**
- Arg151Cys
- Arg160Trp
- Asp294His

“Red Hair Color” phenotype (MC1R-defective)
ANIMAL MODEL
Defective MSH signaling causes fair skin

Dark skin

- Adenylate cyclase
- Mc1<sup>re</sup>E/E
- MSH

Fair skin

- MSH
- Mc1<sup>re</sup>e/e
- Muted cAMP response

Robbins, et. al., Cell, 1993
Human Skin

Melanocytes in hair follicles and basal epidermis

Mouse Skin

Melanocytes only in hair follicles
Stem Cell Factor Signaling

- SCF/c-kit pathway important to melanocyte migration and survival.

- Human basal keratinocytes express c-kit constitutively.

K14 - Stem Cell Factor Transgene

*C57BL/6*

Epidermal melanocytes in the SCF background

Murine model of epidermal melanocytes

**C57BL/6 Tyr<sup>c2j/c2j</sup> Mc1<sup>e/e</sup>**

**C57BL/6 Tyr<sup>+/+</sup> Mc1<sup>e/e</sup>**

**C57BL/6 Tyr<sup>+/+</sup> Mc1<sup>e/E/E</sup>**

K14-SCF<sup>+</sup>

**Eumelanin**

<table>
<thead>
<tr>
<th>Pigment Variant</th>
<th>C2J Albino</th>
<th>Extension</th>
<th>Wild Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/mg dry weight +/− SD</td>
<td>0</td>
<td>500</td>
<td>3000</td>
</tr>
</tbody>
</table>

**Pheomelanin**

<table>
<thead>
<tr>
<th>Pigment Variant</th>
<th>C2J Albino</th>
<th>Extension</th>
<th>Wild Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/mg dry weight +/− SD</td>
<td>0</td>
<td>300</td>
<td>150</td>
</tr>
</tbody>
</table>

D’Orazio, et. al., Nature, 2006
Adaptive melanization is $Mc1r$-dependent

Daily treatments, 5d/wk, one month total.

D’Orazio, et. al., Nature, 2006
Forskolin

- Cell-permeable diterpenoid
- Activator of adenyl cyclase
  - ↑ cytoplasmic cAMP

Coleus forskohlii plant, (Plectranthus barbatus)

http://medicine.osu.edu/news/images/high_quality/Coleus_forskohlii_p1.jpg
Forskolin but not UV rescues eumelanin production in mice with defective MSH signaling.

D’Orazio, et. al., Nature, 2006
Skin Color (L*) +/- SD

Week of study

Topical treatments started
Topical treatments stopped

Forskolin-induced melanin protects against UV damage

Protection against carcinogenesis

A.

B.

<table>
<thead>
<tr>
<th>Tumorigenesis in UV-treated animals</th>
<th>$Mc1^{re/e}$ vehicle</th>
<th>$Mc1^{re/e}$ forskolin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Other tumors</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Multiple tumors</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total tumors</td>
<td>11</td>
<td>6</td>
</tr>
</tbody>
</table>

*9 mice in each group

D’Orazio, et. al., Nature, 2006
Conclusions

- The melanocortin 1 receptor cAMP pathway mediates production of eumelanin by melanocytes.
  - Mc1r defects lead to a fair-skinned, UV-sensitive phenotype.

- Eumelanin production is rescued in an animal model of the fair-skinned human by forskolin.
  - Pigmentary machinery remains intact in Mc1r-deficient state

- Forskolin-induced epidermal eumelanin is highly protective against acute and chronic UV-mediated injury.
  - Novel UV-protective strategy
Mc1r and Melanoma

• Mc1r-defective individuals are at high risk of melanoma.
  – tend to be fair-skinned and UV-sensitive.
  – Having less eumelanin in the skin certainly promotes UV penetration into the basal layer of the epidermis.

• Rate of UV-induced mutagenesis can be affected by rate of production \textit{and} rate of clearance.

• \textit{Could Mc1r signaling also affect melanocyte DNA repair mechanisms?}
Adaptive Pigmentation and DNA Repair in the Skin

UV

Keratinocytes

p53 activation

POMC transcription

Mc1r

Adenylate Cyclase

ATP

cAMP

CREB

PKA

Eumelanin synthesis

DNA repair

Mitf

Pigment Enzymes

β-endorphin

Eumelanin transfer to keratinocytes

α-MSH

ACTH

Cui, et. al., Cell, 2007
Melanoma incidence is on the rise.

- Novel UV- and cancer-protective strategy.
  - safe, healthy tanning
  - better recovery from UV damage!
cAMP modulators in practice

• Methylxanthines
  – Phosphodiesterase inhibition
  – Theophylline
    • Asthma; relaxes smooth muscles in bronchioles
  – Caffeine
    • Stimulant; sed for apnea of prematurity
  – Theobromine
    • Caffeine-like agent in chocolate

• Other phosphodiesterase inhibitors
  – Amrinone, Milrinone
    • Heart failure; positive inotropic effect on heart, vasodilator
  – Rolipram
    • Psychiatric uses; anti-depressant, memory aid, increased wakefulness

Khaled, et. al., 2010, Genes & Development
Thank you!

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