Combinational Effects of Retinoid-X-Receptor α (RXRa) Ablation with Oncogenic Mutations Cdk4^{R24C/R24C} or N-Ras^{Q61K/Q61K} after Ultraviolet Radiation Induced Melanoma Development

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Overview

- Background
- Genetics
- Experimental Design
- Results
- Discussion & Implications
Melanoma

- Leading cause of all skin-cancer related deaths
- 1 in 55 people will be diagnosed in lifetime
- 123,590 new cases of melanoma in 2011
- Risk doubles if person has had 5+ sunburns
Treatment
Dependent on stage
Excision, metastases
Radiation, immunotherapy, chemotherapy

- 5 year survival rate drops from 98% to 15% when melanoma metastasizes
- $249 million/year in treatments for adults 65+
Risk Factors

- Sun exposure, specifically ultraviolet radiation
  Both UVA and UVB
- Artificial tanning
- Moles and fair skin
- Ethnicity
Skin Structure

- Cornified layer
- Granular layer
- Spinous layer
- Basal layer
Effects of UV Radiation

- Killing of pathogens on the skin
- Inducing Vitamin D synthesis
- Treating disorders such as psoriasis vulgaris
- UV:
  - Sunburn
  - Suntan (pigmentation)
  - Epidermal Hyperplasia
  - Immune suppression
  - DNA damage
  - Apoptosis
  - Cell cycle arrest, DNA repair
  - Genetic mutations
  - e.g. in p53, P16/p19, Ras, Raf
- Abnormal cell proliferation
- Photocarcinogenesis (Squamous carcinoma) (Melanoma)
- Skin aging (photoaging)
UV Exposure Mechanism

Initial UV exposure

Paracrine factors to melanocytes

Increase melanin production

Shield the cells from UV radiation
Previous Work

• RXRα has protective roles against carcinogenesis Papillomas and Nevi in the Skin of Mice Selectively Lacking Retinoid-X-Receptor α in Epidermal Keratinocytes (Indra et al, 2007)
• Cell cycles of both keratinocytes and melanocytes affected – crosstalk
• Additive effect with an oncogenic mutation keratinocytes promotes the formation of Cdk4-activated invasive melanomas (Hyter et al, 2010)
Retinoid-X-Receptor α

Binds to promoters of target genes and regulates transcription

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Cdk4\textsuperscript{R24C/R24C}

Eliminates the need for Cdk4 complex to be phosphorylated

Cannot be inhibited by p16

Affects cell cycle progression – mainly transition from G1 to S phase
N-Ras^{Q61K/Q61K}

Ras is a G-protein at the top of the Map Kinase pathway.

Activated form will communicate cell signals to cell’s DNA.

Frequency of 13-25% in all malignant melanomas.

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Keratinocytic RXRα ablation in combination with oncogenic CDK4^{R24C/R24C} or N-Ras^{Q61K/Q61K} will result in an increase in melanocyte proliferation after chronic UV radiation exposure.
Genetics

Experimental Design

N-Ras$^{Q61K/Q61K}$

Cdk4$^{R24C/R24C}$

CT   MT

CT   MT

RXR$^\alpha_{L2/L2}$

RXR$^\alpha_{ep-/-ep-}$
Keratinocytic specific ablation

Flank RXRα with loxP sites (RXRα\textsubscript{L2/L2})

Cross loxP mouse with a K14-Cre mouse

Cre is expressed solely in keratinocytes

Cre recognizes loxP sites, genetic information removed

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## Experimental Design

<table>
<thead>
<tr>
<th>Processing</th>
<th>UV radiation exposure</th>
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<tbody>
<tr>
<td></td>
<td>300mJ/cm² 3 times a week, for 30 weeks</td>
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<table>
<thead>
<tr>
<th>Collection</th>
<th>Punch biopsies taken 20wk, 25wk</th>
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<tbody>
<tr>
<td></td>
<td>Embedded in paraffin, sectioned onto slides</td>
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<tr>
<td></td>
<td>Mice sacrificed 30wk into treatment and final samples collected</td>
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</table>

<table>
<thead>
<tr>
<th>Processing</th>
<th>Fontana-Masson staining</th>
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<tbody>
<tr>
<td></td>
<td>Hematoxylin/Eosin staining</td>
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<tr>
<td></td>
<td>Immunohistochemistry</td>
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<table>
<thead>
<tr>
<th>Data Analysis</th>
<th>Pictures of stained slides</th>
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<tr>
<td></td>
<td>Cell counts done for quantification of proliferating melanocytes</td>
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</table>

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Experimental Design

Processing

FM
melanin

HE
nuclei

IHC
fluorescence

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**Tumor Counts**

**CDK4^{R24C/R24C}**

**Melanoma Formation - 25 weeks Chronic UV**

<table>
<thead>
<tr>
<th>Tumors per Mouse</th>
<th>1-2mm</th>
<th>&gt;2mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDK4^{R24C/R24C}/RXRα^{L2/L2}</td>
<td><strong>2</strong></td>
<td><strong>1</strong></td>
</tr>
<tr>
<td>CDK4^{R24C/R24C}/RXRα^{ep/-}</td>
<td><strong>5</strong></td>
<td><strong>2</strong></td>
</tr>
</tbody>
</table>

P value = 0.05

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Melanin content

20 weeks

RXRα^{L2/L2} / Cdk4^{R24C/R24C}

RXRα^{ep-/-} / Cdk4^{R24C/R24C}

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Ranging Intensities

Control Mouse 124  \((\text{RXR} \alpha^{L2/L2})\)
Ranging Intensities

Ablated MT Mouse 123 (RXRαep−/−)
IHC

Fluorescing antibodies

DAPI  PCNA  TRP1

Nuclei
Proliferation
Melanocyte

Proliferating melanocytes

RXRα

Cdk4^R24C/R24C

Cdk4^R24C/R24C

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Melanomic lesions

20 weeks

\( \text{RXR}^\alpha_{L2/L2} / \text{Cdk4}^{R24C/R24C} \)  \( \text{RXR}^\alpha_{\text{ep}^{-/-}} / \text{Cdk4}^{R24C/R24C} \)
**Pigmentation**

N-Ras\textsuperscript{Q61K/Q61K}

20 weeks

\text{RXR\textalpha}^{L2/L2} / \text{N-Ras}^{Q61K/Q61K}

\text{RXR\textalpha}^{ep/-} / \text{N-Ras}^{Q61K/Q61K}

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IHC

Fluorescing antibodies

DAPI  TRP1

RXRα^{L2/L2} / N-Ras^{Q61K/Q61K}

RXRα^{ep/-} / N-Ras^{Q61K/Q61K}
• RXRα mutation in keratinocytes, together with Cdk4^{R24C/R24C} mutation, enhanced UV irradiation induced formation of melanocytic tumors post chronic UV irradiation.

• Melanocytic tumors from RXRα^{ep-/-} / Cdk4^{R24C/R24C} bigenic mice exhibited increased melanocyte proliferation.
• RXRα mutation in keratinocytes together with N-Ras<sup>Q61K/Q61K</sup> mutation in melanocytes significantly increased formation of dermal melanocytic lesions

• Skin from RXRα<sup>ep-/-</sup> / N-Ras<sup>Q61K/Q61K</sup> bigenic mice exhibited enhanced compaction of melanocytes and melanin in the dermis, and showed increased melanocytes in the epidermis
Discussion

• Hypothesis proven correct – RXRα ablation does increase melanocyte proliferation

• High pigmentation of N-Ras problematic

• Ideally larger sampling would take place

• Qualitative
Future Direction

• Developing a process to bleach melanin out of the dermis and epidermis while maintaining the integrity of the skin sample to be processed through IHC for tumor identification

• Characterization of Cdk4^{R24C/R24C} melanocytic lesions using specific markers for malignant progression (HMB45)

• Continue to process the activated N-Ras samples, focusing on identifying the proliferating melanocytes (PCNA alternative)
Thanks to...

Dr. Arup Indra and Dr. Gitali Indra, as well as Dan Coleman and Stephen Hyter, for their patience and guidance.

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The BioResource Research Program, for introducing me to the wonderful world of research.
Epidermal Thickness

Cdk4^{R24C/R24C}

RXRα L2/L2

RXRα ep/-