Regulation of melanocyte DNA repair by the melanocorticin signaling axis

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Melanoma

• Melanomas arise from malignant transformation of melanocytes

• Melanoma is the deadliest form of skin cancer
  • ~ 87,110 new cases (SEER, 2017)
  • ~ 9,730 deaths
  • Annually, $3.3 billion of skin cancer treatment costs are attributable to melanoma

• Genetic, phenotypic and environmental risk factors all contribute to melanoma predisposition

• UV exposure is a major risk factor
  • Intermittent UV exposure
  • Childhood sunburn

Whereas 10 years ago the risk of developing melanoma was one in 250, today the risk of people getting melanoma is about one in 70.
The melanocortin 1 receptor (MC1R) is a melanocytic transmembrane receptor. It regulates pigmentation and adaptive tanning via cAMP generation. The MC1R gene is highly polymorphic. Loss-of-function MC1R polymorphisms are correlated with melanoma risk. Germline MC1R status influences somatic mutation burden in melanoma (Robles-Espinoza et al., *Nature Communications*. 2016).
The Melanocortin Signaling Axis

UV → keratinocytes → damage response → POMC → MSH

Forskolin → MC1R → adenylyl cyclase → cAMP → MSH-MC1R Signaling Cascade → Reduced DNA Damage

Kadekar et al., Can. Res., 2005
Bohm et al., JBC, 2005

melanocyte
MC1R Signaling Enhances Repair of UV-Induced DNA Damage

[6,4]-photoproducts

Cyclobutane dimers


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**Time Taken to Repair 50% of damage (h)**

- Melanocytes (light pigment)
- Melanocytes (heavy pigment)
- SK-MEL-2
- A375
- MCF-7
- ARPE-19

**Vehicles**

- White bars
- Forskolin bars

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* represents statistical significance.
What are other key proteins involved in the pathway?
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Carol Beach, PhD. Proteomics Core Facility, UK

Kristie Rose, PhD. MSRC Proteomics Laboratory, University of Vanderbilt
A Kinase Anchoring Proteins (AKAPs)

- Family of more > 50 proteins needed for PKA signaling
- Scaffold PKA kinase events
- Regulate PKA localization and activity
  - Brings together PKA with phosphorylation targets and its regulatory proteins

A Kinase Anchoring Protein 12 (AKAP12)

- AKAP12 (also called Gravin and SSeCKS)
  - tumor suppression, cytoskeletal architecture, β₂-adrenergic receptor desensitization/resensitization, cell cycle regulation
  - AKAP12 found at sites of stalled replication forks following nucleotide depletion, however to date, AKAP12 has not been implicated in DNA repair (Sirbu et al., JBC, 2013).
A-Kinase Anchoring Protein 12 (AKAP12) interacts with ATR

- AKAPs scaffold PKA kinase events and localize PKA with phosphorylation targets

ATR-mediated Phosphorylation of AKAP12 Promotes Nuclear Localization

Phosphorylation of AKAP12 at S732 is necessary for nuclear localization

AKAP12

S338 S505 S732 S887 (Matsuoka et al., Science. 2007)

(1,782 amino acids)

NH₂ — COOH

Nuclear localization
(Streb et al., JBC. 2005)

AKAP12 co-localizes with UV-damaged DNA
cAMP enhanced co-localization of XPA-ATR-pS435 requires a functional AKAP12

Topical forskolin application enhances DNA repair in Mc1r-mutant mice

Mc1r

WT Mutant

Translational Implications

Adenylyl cyclase Activators
Phosphodiesterase inhibitors
Melanocortin Analogues

Protective therapy before UV exposure

Enhance repair of UV lesions
Reduced UV mutations
Melanoma Prevention
Summary

- Is ATR-pS435 cell-cycle specific?

- How is the AKAP12-ATR complex transported into and out of the nucleus?

- Does ATR-pS435 impact other DNA repair pathways?

- UV or cisplatin
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