Xeroderma Pigmentosum and the XPA gene

Sarah Drewes
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What is Xeroderma Pigmentosum (XP)?

Extreme sensitivity to UV light

Symptoms:
Distinct freckle-like spots
Severe blistering and burning
Photophobia and eye damage
Cancerous/noncancerous growths
Neurological defects

Why is XP a public health concern?
Nucleotide Excision Repair (NER) is mutated in XP patients!

Mutations in XPA cause XP!

Transcription of downstream proteins affected.

Friedberg, 2001
What is the structure of XPA?

Bartels & Lambert, 2007
What is the structure of XPA?

The most detrimental mutations occur in exons 3, 4, and 5.
**XPA** is well conserved

- **Human** XPA
  - N terminal [DNA Binding] C term
  - %ID: 100%

- **Mouse** XPA
  - N terminal C terminal
  - %ID: 86%

- **Chicken** XPA
  - N terminal C terminal
  - %ID: 65%

- **Drosophila** Xpac
  - N terminal C terminal
  - %ID: 43%

**HomoloGene, InterPro, & BLAST**

= Zinc finger subdomain

Shimamoto et al, 1991
How does XPA function?

Molecular Function

- Protein Binding
- Binding
  - Ion Binding
  - Cation Binding
  - Metal Ion Binding
- Identical Protein Binding
- Heterocyclic Compound Binding
- Organic Cyclic Compound Binding
- Nucleic Acid Binding
  - DNA Binding
  - Damaged DNA Binding
- Protein domain specific binding
Are there compounds that inhibit XPA binding?

Sulforaphane

http://www.dailyperricone.com/2011/02/start-fresh-sulforaphane/
What is sulforaphane’s function?

Oxidative stress response

http://www.grc.nia.nih.gov/branches/lns/linka.htm
How does sulforaphane inhibit XPA?

Sulforaphane removes zinc

Piberger, Köberle, & Hartwig (2014)
Goal: Does the inhibition of XPA by sulforaphane affect other DNA repair processes?

Hypothesis: Sulforaphane will alter binding ability, transcription, and protein expression
Mouse is best model organism to study XPA

Humans and mouse are closely related

http://news.discovery.com/animals/zoo-animals/mice-stem-cells-101209.htm
Mice exhibit similar phenotype as humans

Yamazaki et al, 2005

http://www.atlas-dermato.org/TUMEURS/icono/%20iconoXP.htm
What proteins are involved in causing the XP phenotype in mice?

Proteins have intricate binding network.
XPA and ERCC1 binding is important!

Protein recruiting complex formed
Aim 1: Does sulforaphane eliminate binding between XPA and ERCC1?

Hypothesis: XPA will not be able to bind to ERCC1 in the presence of sulforaphane.
Aim 1: Does sulforaphane eliminate binding between XPA and ERCC1?

Hypothesis: No sulforaphane = transcription ON

Stynen et al, 2012
Aim 1: Does sulforaphane eliminate binding between XPA and ERCC1?

Hypothesis: Sulforaphane present = transcription OFF

Stynen et al, 2012
Experimental conditions for Aims 2 & 3

Wild type + sulforaphane  WT + sulforaphane  XPA mutant + sulforaphane  Mutant + sulforaphane

Does sulforaphane alter...
DNA repair transcripts?
Protein expression over time?
**Aim 2:** Is transcription of DNA repair RNAs affected in the presence of sulforaphane?

**Hypothesis:**

Pre-XPA binding: ↑  
Post-XPA binding: ↓
Aim 2: Is transcription of DNA repair RNAs affected in the presence of sulforaphane?
Aim 3: How does sulforaphane affect DNA repair protein expression levels over time?

Hypothesis: Wild type shows drastic increase in protein expression in response to UV light

Singh et al (2014)
**Aim 3:** How does sulforaphane affect DNA repair protein expression levels over time?

**Hypothesis:** Mutant and wild type + sulforaphane will show decreased expression

Singh et al (2014)
Aim 3: How does \textbf{sulforaphane} affect DNA repair protein expression levels over time?

\textit{Mutant + sulforaphane} will have limited expression and eventual elimination of expression

\textit{Singh et al (2014)}
Conclusions

Sulforaphane inhibits binding partners XPA and ERCC1

Lack of binding alters transcription of DNA repair RNAs

Protein response to DNA damage will decrease over time

What’s next?
Future Research

If binding is inhibited by sulforphane, XP patients should avoid broccoli.

In humans:
Mass spec before and after eating broccoli in XP patients

Hypothesis: Observe modified protein complex + sulforaphane present 🥦
Questions?

http://www.dermrounds.com/photo/xeroderma-pigmentosum-3rd-2?
context=album&albumId=1980062%3AAlbum%3A8589


http://de.academic.ru/dic.nsf/dewiki/1533056

http://buzzkenya.com/interesting-questions-ask-people/