Vitiligo

In vivo and in vitro evidence for epidermal ROS/RNS-mediated regulation / dysregulation

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The skin is the largest outermost organ of the human body

- Size: 1.85-2.00 m²
- Protection of the human body against environmental stress (e.g., radicals, chemicals, heat, cold, water loss etc.)

Outer layer of the human skin

- Keratinocytes
- Melanocytes
- Str corneum
- Str spinosum
- Str basale

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Role of inherited *versus* induced (tanning) skin colour

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The four races of men from the tomb of Sethos I

Inherited skin colour

Skin colour dependent tyrosinase activities

Tyrosinase expression and activity

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Induction of *de novo* melanogenesis by sun – immediate and delayed tanning

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In situ evidence for enhanced pigmentation after sun exposure

Nuclear capping
Melanosomal transfer

Vitiligo – loss of inherited skin and hair colour

Vulgaris
Totalis
Segmental
Inflammatory
Mixed (segmental et vulgaris)

Lips
Ears
Facial
After injury
Pelvis

The burden

“All eyes are looking at me”
From Damned White Spots
(KU Schallreuter, 1999)

Patients have a considerable impaired quality of life...
(Kuiper and Schallreuter et al., 2013)
Correct diagnosis of vitiligo via characteristic fluorescence
Vitiligo vulgaris

Use of Wood’s light for differential diagnosis

e.g., laser-induced leucoderm – No vitiligo

Vitiligo is a disease characterised by an acquired spontaneous loss of the inherited skin colour

• 0.5-2% of the world population are affected
• No significant gender difference
• Positive family history in 35-45%
• Onset at any time in life (more frequent in puberty, pregnancy, menopause)
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The cause of vitiligo is yet unknown!!!

Pathogenesis - not conclusive

- Autoimmune?
- Neurological?
- Self destruction of melanocytes (autocytotoxic)?
- Oxidative stress?
- Biochemical abnormalities?
- Genetic?
- Virus?
- Hormones?

Patients with vitiligo accumulate millimolar concentrations of H$_2$O$_2$ in their epidermis

Acute facial vitiligo (clinical picture)

Schallreuter et al., 1998; Schallreuter KU, 2005; Schallreuter et al., 2007; 2008; Schallreuter et al., 2009; Schallreuter et al., 2012
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Evidence for epidermal H$_2$O$_2$ (mM) in vitiligo by in vivo FT-Raman spectroscopy

Exogenous H$_2$O$_2$ - sources

- Solar exposure
- Chemical compounds (e.g., hydroquinone, Q10, xenobiotics etc.)
- Hormones (e.g., estrogens, progesterons etc.)

Depigmentation after hydroquinone treatment

Chemical induced vitiligo

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**Depigmentation after topical Q10 treatment**

Chemical induced vitiligo

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**Intrinsic H₂O₂ sources in epidermal cells**

- Xanthine oxidase
- Quinones
- Estrogens/progesteron
- Polymersation of melanin
- TGFβ/FGF
- EGF
- Photo-oxidation of planns
- NTRF
- SOX
- NADPH-oxidase
- Monoamine oxidase A

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**Evidence for lipid peroxidation**

in epidermal keratinocytes and melanocytes

in untreated vitiligo

- Bhawan and Butami 1983
- Tobin et al., 2000

H₂O₂ + fatty acids

→ lipid peroxidation

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Epidermal H$_2$O$_2$-mediated stress leads to oxidation of:

• Methionine
• Tryptophan
• Cysteine
• Seleno cysteine residues in protein sequences

Resulting in partial or complete loss of functionality and deactivation of affected proteins

In vivo FT-Raman spectroscopy confirms epidermal H$_2$O$_2$-mediated oxidation in vitiligo

H$_2$O$_2$ affects

• Entire cofactor 6-tetrahydrobiopterin (6BH$_4$) biosynthesis and recycling, consequently affecting with epidermal catecholamine, serotonin and melatonin synthesis

Schallreuter et al., 1994, Marks et al., Schallreuter et al., 2012
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The clinical hallmark of vitiligo – Fluorescence of biopterins under Wood’s light

Schallreuter KU et al., Science, 1994

H₂O₂ oxidises 6BH₄ and its 7- isomer to 6-bioperin and 7-bioperin

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Direct effect of \( \text{H}_2\text{O}_2 \) on 6BH\(_4\) recycling via PCD

L-phenylalanine → Phenylalanine hydroxylase → L-tyrosine

GFRP → 6BH\(_4\) → 4a-OH-BH\(_4\) → 7BH\(_4\)

Sepiapterin reductase → 6-Pyruvoyl-PH\(_2\)-synthase → GTP-cyclohydrolase I → GTP

\( \text{H}_2\text{O}_2 \) affects 6BH\(_4\) recycling via PCD

Low epidermal PCD activity

Epidermal PCD protein expression

\( \text{H}_2\text{O}_2 \) affects epidermal pro-opiomelanocortin - processing and signalling
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Cleavage of pro-opiomelanocortin by prohormone convertase 1 and 2/7B2

Evidence for H₂O₂-mediated oxidation of epidermal α-MSH and β-endorphin in vitiligo

Oxidised α-MSH and β-endorphin lose their function in epidermal melanocyte
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What about the antioxidant defence machinery?

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Evidence for oxidation/deactivation of important epidermal antioxidant enzymes by H$_2$O$_2$

- Catalase
- Thioredoxin reductase/thioredoxin
- Glutathione reductase/glutathione
- Glutathione peroxidase
- Methionine sulfoxide reductase A/B

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H$_2$O$_2$ decreases catalase protein expression and enzyme activities in the human epidermis

Schallreuter et al., 2008; Wood et al., 2008
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37
et al., 1991; Gibbons et al., 2006

H$_2$O$_2$ deactivates epidermal catalase in vitiligo due to oxidation of Met and Trp residues

38
et al., 2006

H$_2$O$_2$-mediated oxidation affects NADPH - binding of catalase

39
et al., 1987; 1991; 2008; Gibbons et al., 2006

Epidermal thioproteins are severely affected in vitiligo

Thioredoxin reductase

H$_2$O$_2$ decreases epidermal thioredoxin reductase protein expression and activities in vitiligo
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H$_2$O$_2$-mediated oxidation in vitiligo affects

Epidermal methionine sulfoxide repair via methionine sulfoxide reductase A and B

Repair of R&S methionine sulfoxide diastereomers by MSR A & B

MetS=O + 2NADPH → Met + 2NADP$^+$

Decreased epidermal MSRA and MSRB enzyme activities in vitiligo

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Structural modelling of MSRA supports \( \text{H}_2\text{O}_2 \)-mediated deactivation of the enzyme active site

Overlay of native (green) and oxidised enzyme (red)

Evidence for epidermal peroxinitrite production in vitiligo

\[
\text{NO} + \text{O}_2^- \rightarrow \text{ONOO}^-
\]

Presence of high epidermal peroxynitrite (ONOO\(^\cdot\)) levels in vitiligo

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Evidence for increased S-nitrosylation in vitiligo

H₂O₂/ONOO⁻ accumulation in vitiligo leads to DNA damage

Evidence for DNA damage (8-oxyguanine) in the epidermis of patients with vitiligo
Evidence for DNA damage (8-oxoguanine) in plasma of patients with vitiligo

*4.0
2.5
2.0
1.5
1.0
0.5
0.0

Controls (n=10) Patients (n=20)

Epidermal H₂O₂ is transferred to the system

Hasse et al., 2004; Schallreuter et al., 2008; 2013

Evidence for systemic oxidative stress in vitiligo - epidermal H₂O₂ affects systemic enzymes

Recovery after reduction of epidermal H₂O₂ with a topical pseudocatalase PC8KUS

DHPR activity in whole blood

Hasse et al., 2004
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\[ \text{H}_2\text{O}_2 \text{ affects epidermal tryptophan metabolism via:} \]

- Tryptophan hydroxylase, serotonin/melatonin balance
- Indoleamine 2,3-dioxygenase (IDO) induced immune response
- Arylhydrocarbon receptor (ARH) signaling

\[ \text{H}_2\text{O}_2 \text{ affects epidermal tryptophan metabolism via:} \]

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\[ \text{H}_2\text{O}_2 \text{ affects epidermal tryptophan metabolism via:} \]

- Tryptophan hydroxylase, serotonin/melatonin balance
- Indoleamine 2,3-dioxygenase (IDO) induced immune response
- Arylhydrocarbon receptor (ARH) signaling

**Consequences of epidermal tryptophan shortage in vitiligo**

Impaired immune response via epidermal indoleamine 2,3 dioxygenase and aryl hydrocarbon receptor signaling
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**In summary**

- In vitiligo overproduction of epidermal $\text{H}_2\text{O}_2$, NO and ONOO$^-$ affects:
  - ROS defense
  - Melanogenesis
  - Neuroendocrine response
  - Calcium signalling
  - T-cell response

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**The paradox in vitiligo**

- DNA damage but no evidence for increased incidence of solar induced skin cancer
  - Calanchini-Postizzi & Frenk 1989
  - Schallreuter et al., 2002
  - Teufling et al., 2013

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Epidermal p53<sup>wt</sup> is up-regulated and functional in vitiligo

Increased p53 in epidermal cell extracts in vitiligo

Western blot

Increased p53 in epidermal MC from lesional vitiligo

Control melanocytes

Vitiligo melanocytes

Increased p53 in epidermal cell extracts in vitiligo

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Can we do anything?

Reduction of epidermal $H_2O_2$ with low dose NB-activated pro-pseudocatalase PC-KUS

Up-regulation of epidermal catalase protein expression by $H_2O_2$ (µM)

Control $H_2O_2$ (mM) $H_2O_2$ (µM)
**Vitiligo**

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**Up-regulation of epidermal thioredoxin reductase protein expression by $\text{H}_2\text{O}_2$ (µM)**

- Control
- Vitiligo
  - $\text{H}_2\text{O}_2$ (mM)
  - $\text{H}_2\text{O}_2$ (µM)

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**Reduction of $\text{H}_2\text{O}_2$ increases epidermal β-endorphin expression**

- Control
- Repigmenting
  - Lesional
  - Ab blocking

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**Reduction of $\text{H}_2\text{O}_2$ increases epidermal α-MSH expression**

- Control
- Repigmenting
  - Lesional

---

*Spencer et al., JID 2007*
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In summary - H$_2$O$_2$ has two sites

- At high concentrations: loss of functionality of peptides and enzymes
- At low concentrations: stimulation of peptide and enzyme activities

From the bench to the bedside

Reduction of epidermal H$_2$O$_2$

by activated pro-pseudocatalase PC-KUS

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Recovery of the skin colour after epidermal H$_2$O$_2$ reduction

47 years generalised vitiligo

2 years segmental vitiligo

Recovery of facial vitiligo after reduction of epidermal H$_2$O$_2$

with activated pro-pseudocatalase PC-KUS

Woods light documentation

Recovery of facial vitiligo after reduction of H$_2$O$_2$

with pro-pseudocatalase PC-KUS

Before

4 month later

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Repigmentation after reduction of epidermal H$_2$O$_2$ with activated pro-pseudocatalase PC-KUS

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Recovery of the skin colour after epidermal H$_2$O$_2$ reduction with activated pro-pseudocatalase PC-KUS

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Vitiligo of the lips and ears after reduction of epidermal H$_2$O$_2$ with activated pro-pseudocatalase PC-KUS
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**No influence of inherited skin colour on repigmentation in vitiligo**

![Graph showing no influence of inherited skin colour on repigmentation in vitiligo](image)

- Recovery of affected proteins and peptides
- Cessation of disease progress
- Recovery of skin colour

**Reduction of epidermal H$_2$O$_2$ by activated pro-pseudocatalase PC-KUS yields:**

- Recovery of affected proteins and peptides
- Cessation of disease progress
- Recovery of skin colour

**What did we learn?**

1. Vitiligo affects both, epidermal melanocytes and keratinocytes
2. In vivo and in vitro evidence for H$_2$O$_2$/ONOO$^-$ accumulation and H$_2$O$_2$/ONOO$^-$ mediated oxidation and nitration of proteins and peptides in the entire epidermal compartment of affected individuals
3. Evidence for DNA damage in the epidermis and plasma of patients – but no increased risk for solar induced skin cancer
4. Effective DNA repair in association with constant up-regulated wild type functioning p53 and up-regulated MDM2 p76
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Vitiligo - The dilemma

The hope – complete recovery of the skin colour

"Turning white"
by Lee Thomas

Turning black
3 months after $\text{H}_2\text{O}_2$ reduction with pro-pseudocatalase PC-KUS

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