Basic mechanisms of chemotherapy-induced alopecia (CIA)

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For the record
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Sudden, cancer therapy-associated massive hair loss

A massive psychoemotional Stressor!

Sudden, cancer therapy-associated massive hair loss
Stress
Hair loss
Depression Anxiety
Self-perception
Coping
Society
Education

Stress management strategies
CNS-targeting drugs
Psychological and behavioural therapy
Cosmetic treatment (wigs et c.)

Therapeutic Implications:

Therapy refusal!

CRH, ACTH, Cortisol, Prolactin, SP, NGF

Hadshiew et al.
J Invest Dermatol 2004
Most amazing mini-organ that evolution has come up with
„All the beef is in the bulb“

Hair follicle
Anagen hair bulb = hair shaft factory

Chemotherapy

HF pigmentary unit

Dermal papilla „The boss“

Hair matrix
Hair follicle

ca. 5 million mini-organs
scalp: ca. 100,000 HFs

„work horse of the skin“

→ fiber & pigment production
→ epidermal regeneration & repigmentation

→ Important for wound healing, angiogenesis, innervation etc.pp.

→ Major hormone & protein factory

→ highly sensitive to nutrients, hormones, „stress“, drugs

Unique feature:

Cyclic organ remodelling
HF = regeneration miracle & anti-aging wonder

- HF cycles "forever"
- multiple stem cell pools

• EXCEPTION: Greying!
  Aging of HF pigmentary unit & HF melanocyte stem cells

→ ONE organ to study both, aging & anti-aging

→ Chemotherapy promotes tissue aging!
**HF = regeneration miracle & anti-aging wonder**

- HF cycles „forever“
- multiple stem cell pools
- exc. DNA repair
- telomerase
- ROS scavenging enzymes
- antioxidants
  - Haslam et JID 2016
  - melatonin
  - Kobayashi et al. FASEB J 2005

**EXCEPTION: Greying!**
Aging of HF pigmentary unit & HF melanocyte stem cells

Harbors a neuroendocrine stress-response system
CRH \(\rightarrow\) ACTH \(\rightarrow\) cortisol
Prolactin, NGF, subst P
Responds to psychoemotional & chemical stressors
\(\rightarrow\) Hair growth inhibition
Cancer therapy-associated hair loss: Key frontier in psychooncology

• Sudden loss of hair as a key instrument of mammalian communication → CIA= profound psychoemotional stressor → acute & chronic stress responses + depression

• Psychoemotional „stress“ is hair growth-inhibitory!
  → Neurogenic perifollicular inflammation (NGF→ substance P→ mast cells activation) induces premature catagen and attacks HF stem cells (bulge)

• Hair follicles are targets & sources of key stress mediators: CRRH→ACTH→cortisol, PRL → inhibit hair growth!
  → Enhanced intrafollicular generation of hair growth-inhibitory stress hormones by chemotherapy & CIA-associated stress?

→ Psychological stress intervention: essential in CIA on multiple levels

Why relevant in CIA?

- HF cycles „forever“
- multiple stem cell pools
- exc. DNA repair
- telomerase
- ROS scavenging enzymes
- antioxidants
- Haslam et JID 2016
- melatonin
  Kobayashi et al. FASEB J 2005

Scalp cooling ???

Future CIA management vision:

→ Upregulate the above before/during/after chemotherapy by HF-targeting topical agents
→ Dampen HF stress response systems
Chemotherapy-induced alopecia (CIA) = hair cycle disruption

Paus et al. *Lancet Oncol* 2013
Premature catagen induction: Telogen effluvium

Telogen = club hairs depigmented tip

Dysruption of anagen without normal catagen induction

Anagen = "broom stick" IRS+ pigmented tip

CIA

Anagen effluvium
Chemotherapy-induced alopecia (CIA)

Reversibility of CIA depends on stem cell survival

Vision: Reduce permanent CIA by administering topical stem cell protectants

* e.g. PPARγ agonists, spermidine, endocannabinoids
human anagen hair bulb

**hair follicle pigmentary unit**
- differentiated melanocytes

**connective tissue sheath**
- fibroblasts, mast cells, macrophages, endothelial cells
- progenitor cells
  - pericytes
  - nestin+ cells
  - Schwann cells

**outer root sheath**
- keratinocytes
- amelanotic melanocytes/-blasts
- T cells, Langerhans cells

**hair matrix**
- massively proliferating keratinocytes

**follicular dermal papilla**
- inductive fibroblasts

**Most sensitive to noxious stimuli/agents**
Most sensitive to noxious stimuli/agents

Conventional wisdom:

Chemotherapy →

Cycling hair matrix keratinocytes

p53 → apoptosis

human anagen hair bulb

hair matrix
massively proliferating keratinocytes
Intraperitoneal injection of high-dose cyclophosphamide into adolescent/adult, darkly pigmented C57BL/6 mice with all back skin HFs in depilation-induced, early anagen VI causes massive hair matrix apoptosis, HF dystrophy and rapid alopecia, perfectly imitating human cyclophosphamide-induced alopecia.


Murine CIA model

Human pendant:
Scalp HF organ culture
• **p53** plays a key role in mediating CYP-induced massive hair matrix apoptosis and HF dystrophy

• Mediated via **Fas** (=direct p53 target!)?

Sharov et al. *Cancer Res* 2004
Human scalp HF organ culture
→ identify hair growth promoters & inhibitors

1 Anagen-catagen switch

CATAGEN

Catagen inducers
= Hair growth inhibitors

Catagen inhibitors
= hair growth promoters

ANAGEN VI

2 Hair shaft elongation

3 Gene & protein expression profile

4 Hair matrix proliferation / apoptosis, pigmentation

5 pigmentation

6 Progenitor cells

7 Mast cells / Macrophages

8 Gene silencing possible!

Use to test impact of chemotherapy
Cyclophosphamide (4-HC) severely disrupts HF pigmentation:

- Ectopic melanin
- Melanin clumping

→ Sensitive parameter for quantification of human HF damage level

Cyclophosphamide metabolite (4-HC) induces a distinct and reproducible "gene expression damage profile" of human HFs

- Define molecular damage-response profile of any chemotherapeutic agent in a living human organ!
- Candidate anti-CIA agents: "protection fingerprint"
- scalp cooling ???
HF damage responses to chemotherapy

**ORS:** TGFβ1/2 up, IGF-1 down, CRH up reduced mitochondrial activity; loss of epithelial & melanocyte progenitor cells

**CTS:** Mast cells degranulate, MACs activated

Disruption of HF pigmentation:
Ectopic melanin, melanin clumping, Cessation of melanogenesis

**Hair matrix keratinocytes:**
Increased apoptosis, reduced Proliferation; p53 up,
Increased oxidative & DNA damage

Faster emigration of and reduced morphogen production by DP cells

→ Premature catagen, stop of hair shaft production

LDH, IL-8, IL-6, TNF-α release up
Conventional wisdom:

Chemotherapy →

Cycling hair matrix keratinocytes

p53 → apoptosis

Bodo et al. AJP 207

Shh downregulation

Xie / Paus / Yie J Invest Dermatol 2015
& unpublished

hair matrix apoptosis

→ Vismodegib

→ scalp cooling ???
Figure 6. Loss of Shh signalling is associated with CIA and hair follicle dystrophy (A). HFs were plucked from patients undergoing chemotherapy (data provided by ZY, China). Ahh gene expression was down regulated in all patients in which hair loss was evident. (B) Human HFs were cultured in the absence (Control) or presence of the Shh pathway inhinitor cyclopamine for 48 hours. Immunostaining showed an decrease in proliferative (Ki67+) and an increase in apoptotic (TUNEL+) matrix keratinocytes. Staining by Masson Fontana histochemistry showed melanin clumping (blue arrows), indicative of dystrophic catagen. These results indicate that loss of Shh signaling is correlated with hair loss in response to chemotherapy in vivo and that ex vivo disruption of the pathway can induce the HF dystrophy normally associated with CIA.

Shh mRN downregulated in plucked hair shafts from all patients with CIA

Haslam/Paus/Yue, unpublished

Cylophosphamide or Shh silencing both Inhibit proliferation & Induce apoptosis in human hair matrix

...and so does cyclopamine

CIA management vision: Up-regulate Shh expr/activity in hair matrix
Does chemotherapy up-regulate SFRP1-secretion from the DP?

Cyclosporine A cause hypertrichosis, inhibits catagen
Hawkshaw et al. JID 2015

Cyclosporine A also
Inhibits CIA in mice
Paus et al. AJP 1994 + 1997

Cyclosporine A suppresses SFRP1
Hawkshaw et al. PLoS Biol 2018

Shh → p53 → apoptosis

SFRP1

follicular dermal papilla (DP)
inductive fibroblasts

Hawkshaw et al. PLoS Biol 2018

Does chemotherapy up-regulate SFRP1-secretion from the DP?

WAY-316601 suppresses SFRP1
CIA

Dystrophic anagen

Dystrophic catagen

Pigmentary abnormalities = very sensitive dystrophy marker!

Hendrix et al. J Invest Dermatol 2005
Paus et al. Lancet Oncol 2013
Mice in vivo

Chemotherapy-induced alopecia

**dystrophic anagen pathway**

- CsA, FK506, IL-15-FP, PTH/PTHrP 7-34

**CYP**

- hair shaft shedding
- primary recovery
- secondary recovery

**Calcitriol, Glucocorticoids, PTH 1-34**

**dystrophic catagen pathway**

- hair shaft shedding
- shortened dystrophic telogen
- secondary recovery
Conundrum:

• Path to fastest & best hair recovery shows the most massive initial alopecia

• Does it even make sense to inhibit CIA?
Mice in vivo

CsA, FK506, IL-15-FP, PTH/PTHrp 7-34

CYP

Doxorubicin

Calcitriol, Glucocorticoids, PTH 1-34

17-ß estradiol

Human HF ex vivo

CIA

dystrophic anagen pathway

hair shaft shedding

primary recovery

secondary recovery

EPO

α-MSH

Melatonin

KGF (FGF7)

Shh up-

Regulation?

SFN?

17-ß-estradiol

Prednisolone (Vit D: calcitriol)
Generate "multi-drug resistant" HFs

Topical ABC transporter inducing agent

- ABC transporter expression
- Bulge stem cells
- Proliferating matrix keratinocytes

Multidrug resistant HFs

Protection against CIA: targeting ABC transporters

Reduced intracellular drug accumulation in target cell

ABC transporter
Chemotherapy drug

### Table: ABC Transporter Expression in ORS Keratinocytes

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Agonist</th>
<th>Concentration</th>
<th>ABCB1</th>
<th>ABCC1</th>
<th>ABCC3</th>
<th>ABCC4</th>
<th>ABCG2</th>
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<tbody>
<tr>
<td><strong>PPARγ</strong></td>
<td>Rosiglitazone</td>
<td>10µM</td>
<td>1.8±0.2</td>
<td>1.0±0.0</td>
<td>1.0±0.1</td>
<td>0.8±0.0</td>
<td>0.8±0.0</td>
</tr>
<tr>
<td><strong>GR</strong></td>
<td>Dexamethasone</td>
<td>50µM</td>
<td>2.1±0.6</td>
<td>1.0±0.0</td>
<td>1.2±0.2</td>
<td>1.0±0.1</td>
<td>1.6±0.5</td>
</tr>
<tr>
<td><strong>VDR</strong></td>
<td>Vitamin D</td>
<td>0.5µM</td>
<td>17.1±3.8</td>
<td>1.3±0.1</td>
<td>1.5±0.2</td>
<td>0.6±0.0</td>
<td>2.9±0.4</td>
</tr>
</tbody>
</table>

**Primary human HF keratinocytes (ORS Kc) express key ABCs**

**Figure 4.** ABC transporters are expressed in ORS keratinocytes. The graph shows the expression of ABC transporters, relative to the housekeeping gene PPIA, comparing ORS keratinocyte expression levels with that of Caco-2 cells, a commonly used intestinal cell line known to express the transporters of interest. ABC2, G2 and C4 show lower expression levels in ORS keratinocytes than Caco-2 cells, whereas ABCC1 and C3 are comparable.

**Induction of ABC transporters in human ORS keratinocytes** (24 h, mRNA, qRT-PCR)
Don‘t just accept this:

Do something!
We can & must do better than just scalp cooling e.g. explore

How does scalp cooling work ???

EPO, $\alpha$-MSH
Melatonin, KGF

Up-regulate HF defenses against oxidate damage
Sulforaphane /Nrf2

Enhance intra-HF DNA repair & telomerase activity

Upregulate Shh
Downregulate SFRP1

Stem cell protectants
Cell cycle arrest

Make HFs „multi-drug resistant“

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