Use of gene silencing in skin models

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Why gene silencing in an organotypic skin model?

- It would enable the study of gene deletions in a complex *in vitro* system of human cells.
- We would be able to investigate a direct involvement of target genes in skin development/differentiation, without the influence of other cell types (cells of the immune-system).
- It would strongly reduce the necessity of animal experiments in dermatological research.
- It would reduce time and costs compared to a knock out animal approach.
Organotypic skin model

- Differentiating human epidermal keratinocytes
- Growth on a "dermis-like" support
- Simultaneous analysis of the different steps of terminal KC differentiation
- Studies of the alterations induced by chemicals, pharmaceuticals on this differentiation program
Morphology of the organotypic skin

Organotypic skin
Methodology for gene silencing

- Lipofection of siRNA
- Monolayer culture
  - DMEM with 10% FBS
  - 48 hours post transfection
- Organotypic skin culture
  - Matrigel
  - 7 days

Differentiation in monolayer culture

- Morphology: H&E
- Immunofluorescence
- Western blot
- RT-PCR
Gene knockdown by short interfering RNA (siRNA)

- **siRNA oligonucleotides**
- **transfection with lipofectamin**
- **sequence-specific binding**
- **mRNA cleavage by DICER**
- **non-functional mRNA fragments**

**siRNA, general structure**

- 19-nt duplex
- 2-nt overhangs
<table>
<thead>
<tr>
<th><strong>VEGF conditional KO-mouse</strong></th>
<th><strong>Matriptase-1 KO-mouse</strong></th>
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<td>• No influence on the development of the epidermis</td>
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<td>Ref.: Rossiter H et al, Cancer Res. 2004 May</td>
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• Mice die 48 hours after birth, due to severe skin problems
• Defect in lipid matrix formation, cornified envelope morphogenesis and stratum corneum desquamation
• Loss of processed filaggrin monomer and filaggrin S-100 protein
• Accumulation of pro-filaggrin
• Transplanted skin shows an ichthyosis like phenotype

Ref.: List K et al, Oncogene. 2002 May
Ref.: List K et al, J Cell Biol. 2003 Nov
VEGF deficient skin equivalents
VEGF conditional KO-mouse

normal development of the epidermis

Ref.: Rossiter H et al, Cancer Res. 2004 May
VEGF siRNA in organotypic skin cultures

untreated  mock  CD4

VEGF siRNA 1  VEGF siRNA 2

86%  75%
Matriptase-1 deficient organotypic skin cultures
Matriptase-1 KO mouse

Severe skin problems

Transplanted skin

H&E staining

Matriptase-1 siRNA knock down in organotypic skin cultures
Filaggrin processing

Impaired filaggrin processing

- untreated
- mock
- CD4
- matriptase-1 siRNA 1
- matriptase-1 siRNA 2

Pro-filagrin

53 kDa accumulated filagrin
Summary

• Organotypic skin cultures of Matriptase-1 and VEGF siRNA treated keratinocytes show comparable results to the corresponding KO-mice.

• Organotypic skin cultures of keratinocytes transfected with VEGF siRNA show:
  • no phenotype

• Organotypic skin cultures of keratinocytes transfected with Matriptase-1 siRNA show:
  • Hyperkeratosis
  • Parakeratosis
  • Impaired filaggrin processing
DNase1L2 degrades nuclear DNA during corneocyte formation

Heinz Fischer, Leopold Eckhart, Michael Mildner, Karin Jaeger, Maria Buchberger, Minoo Ghannadan, Erwin Tschachler

Structure of the epidermis
Nuclear DNA is degraded during formation of the cornified layer
Hypotheses

1. During keratinocyte differentiation nuclear DNA is degraded by a specific DNase.

2. The expression of this DNase is upregulated during keratinocyte differentiation.
Keratinocyte differentiation is associated with upregulation of DNase1L2 mRNA.
DNase1L2 is predominantly expressed in skin
DNase1L2 is expressed in differentiated epidermal keratinocytes.

- **Epidermis**
- **Hair follicle**
- **Sebaceous gland**

**Anti-DNase1L2**

**Anti-DNase1L2 + rec. DNase1L2**
In skin with parakeratotic stratum corneum DNase1L2 expression is reduced

Immunofluorescence: anti-DNase1L2
Knockdown of DNase1L2 in skin equivalents is highly efficient
Knockdown of DNase1L2 in skin equivalents results in parakeratosis

siRNA: control

siRNA: DNase1L2

anti-DNase1L2

anti-loricrin
Knockdown of DNase1L2 in skin equivalents results in parakeratosis

siRNA: control  siRNA:DNase1L2
Summary: DNase1L2

- specifically expressed in the epidermis
- expression correlates with keratinocyte differentiation
- absence correlates with parakeratosis in psoriasis
- specific knockdown of DNAse1L2 by si RNA technology in SE culture results in retention of nuclei in corneocytes
Conclusion

DNase1L2 is essential for the degradation of nuclear DNA during stratum corneum formation
Other Genes examined so far:

- JunB
- Filaggrin
Transcription of the caspase-14 gene in human epidermal keratinocytes requires AP-1 and NFκB

Claudia Ballaun, Susanne Karner, Paul Mrass, Michael Mildner, Maria Buchberger, Jürgen Bach, Jozef Ban, Hanna Harant, Erwin Tschachler and Leopold Eckhart

JunB knock down led to an altered epidermal architecture with an irregular stratification, a reduced or absent granular layer and the appearance of foamy cells and vacuoles in the suprabasal layers.
(Pro)filaggrin knock out in an organotypic skin model
Filaggrin knock-down increases sensitivity to UVB-irradiation

- **No treatment**
  - Untreated
  - FLG siRNA2
  - FLG siRNA3

- **UVB 100 mJ/cm²**
  - Untreated
  - FLG siRNA2
  - FLG siRNA3

- **UVB 150 mJ/cm²**
  - Untreated
  - FLG siRNA2
  - FLG siRNA3
Potential of this technique

• It enables the study of deletion of individual genes in a complex system (cell-cell interaction, cell-matrix interaction,...) of human cells.
• This technique could strongly reduce the necessity of animal experiments in dermatological research.
• The *in vitro* knock down is less expensive and much less time consuming than animal models.
Improvement of this model

• Generation of organotypic skin cultures containing other cell-types of the skin
  – Melanocytes
  – Langerhans cells
  – Microvascular endothelial cells

• Generation of organotypic skin tumors
  – Squamous cell carcinoma
  – Basal cell carcinoma
  – Melanoma
Acknowledgements

Claudia Ballaun
Jiang Jin
Heinz Fischer
Leopold Eckhart
Reinhard Bauer
Martin Stichenwirth
Maria Buchberger
Ramona Gmeiner
Veronika Mlitz
Minoo Ghannadan
Arby Abtin
Heidi Rossiter
Erwin Tschachler