New tracks into the pathophysiology of Peeling Skin Disease revealed by transcriptomic analysis of two *Corneodesmosin*-deficient mouse models

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BACKGROUND

- Peeling skin disease (PSD, OMIM 270300) is a rare **autosomal-recessive inflammatory ichthyosis** due to homozygous loss-of-function mutations in *Corneodesmosin* (*CDSN*) and characterized by **lifelong patchy peeling of the skin** associated with **eczema, food allergy** and **severe itching**. It represents a **serious handicap** and **no specific therapy** is currently available.

(Oji et al., Am. J. Hum. Genet., 2010a)

- CDSN* is an **epidermal adhesive protein** specific to **corneodesmosomes** which plays a crucial role in the ***stratum corneum* cohesion and desquamation**.

CDSN in human epidermis
(Serre et al., J. Inv. Derm., 1991)

Impaired permeability barrier

Epidermis-specific *Cdsn* KO neonates: lethal phenotype
(Leclerc et al., J. Cell. Sci., 2009)

Split desmosomes (arrows) at the transition *stratum granulosum/stratum corneum*

Ultrastructure of KO epidermis

AIMS

The initial event of PSD, the detachment of the *stratum corneum* due to CDSN deficiency, leads to an impairment of the permeability barrier. This defect could in turn trigger erythema, pruritus and atopic manifestations by so far unidentified mechanisms. In order to **explore these pathophysiological mechanisms**, we analyzed **cutaneous gene expression profiles** of two epidermal-specific *Cdsn*-deficient mouse models representative of **early and chronic phases of the disease**.

MOUSE MODELS

“Early” PSD mouse model

Stratum corneum detachment: occurrence of the permeability defect

Non-stripped skin Stripped skin

“Chronic” PSD mouse model

Epidermis-specific inducible *Cdsn* KO adult mice (*Cdsn*^{EP-/-}):
Daily application of hydroxytamoxifen on ears (15 days)

Topical induction of *Cdsn* deficiency: permanent permeability defect

RESULTS

1- “Early” PSD mouse model: gene expression profiles in the skin

Number of differentially expressed genes (in comparison with control embryos)

p-value ≤ 0.05	Down-regulated genes	Up-regulated genes
Fold Change (FC)	≤ -1.4	≥ 1.4
Number of genes	1487	3973

Top 3 of functions corresponding to genes strongly up-regulated (FC ≥ 4)

Functions	Gene Symbol	Gene Description	FC
Cytokine activity	<i>Cxcl5</i>	Chemokine (C-X-C motif) ligand 5	17.8
	<i>Il1b</i>	Interleukin 1 beta	7.8
	<i>Cxcl3</i>	Chemokine (C-X-C motif) ligand 3	5.4
	<i>Ccl20</i>	Chemokine (C-C motif) ligand 20	5.3
	<i>Ccl2</i>	Chemokine (C-C motif) ligand 2	5.0
	<i>Il24</i>	Interleukin 24	4.7
	<i>Il6</i>	Interleukin 6	4.4
Growth factor activity	<i>Ifi202b</i>	Interferon activated gene 202B	4.3
	<i>Lif</i>	Leukemia inhibitory factor	4.2
	<i>Ifnk</i>	Interferon kappa	4.0
Growth factor activity	<i>Ereg</i>	Epiregulin	8.0
	<i>Fgf23</i>	Fibroblast growth factor 23	7.4
	<i>Epgn</i>	Epithelial mitogen	6.0
	<i>Ngf</i>	Nerve growth factor	5.6
	<i>Areg</i>	Amphiregulin	5.5
Cornified envelope cross-linking	<i>Spr2g</i>	Small proline-rich protein 2G	7.4
	<i>Lce3d</i>	Late cornified envelope 3D	5.9
	<i>Spr2i</i>	Small proline-rich protein 2I	5.4
	<i>Spr2e</i>	Small proline-rich protein 2E	4.2
	<i>Lce3f</i>	Late cornified envelope 3F	4.1

¹also growth factor activity
FC: Fold Change

Predominant biological processes according to the list of the 3973 up-regulated genes

Validation by RT-PCR

Up-regulation of inflammatory and proliferative genes

2- “Chronic” PSD mouse model: gene expression profiles in the skin

Number of differentially expressed genes (in comparison with control mice)

p-value ≤ 0.05	Down-regulated genes	Up-regulated genes
Fold Change (FC)	≤ -1.4	≥ 1.4
Number of genes	834	2577

Top 3 of functions corresponding to genes strongly up-regulated (FC ≥ 4)

Functions	Gene Symbol	Gene Description	FC
DAMP/Alarmin	<i>S100a9</i>	S100 calcium binding protein A9	37.3
	<i>S100a8</i>	S100 calcium binding protein A8	16.2
	<i>Defb4</i>	Defensin beta 4	11.9
	<i>Lcn2</i>	Lipocalin 2	5.0
	<i>Stfa3</i>	Stefin A3 (cystatin A)	34.1
Protease/Protease inhibitor	<i>Tpsab1</i>	Tryptase alpha/beta 1	19.7
	<i>Klk13</i>	Kallikrein related-peptidase 13	13.1
	<i>Stfa1</i>	Stefin A1 (cystatin A)	11.8
	<i>Adam8</i>	A disintegrin and metallopeptidase domain 8	4.5
	<i>Stfa2</i>	Stefin A2 (cystatin A)	4.2
Cornified envelope cross-linking	<i>Spr2a2</i>	Small proline-rich protein 2A2	16.8
	<i>Lce3f</i>	Late cornified envelope 3F	14.1
	<i>Spr2e</i>	Small proline-rich protein 2E	12.4
	<i>Lce3e</i>	Late cornified envelope 3E	11.9
	<i>Spr2d</i>	Small proline-rich protein 2D	11.2
	<i>Spr2a2</i>	Small proline-rich protein 2A2	10.2
	<i>Rptn</i>	Repetin	10.0
	<i>Spr2g</i>	Small proline-rich protein 2G	7.9
	<i>Spr2h</i>	Small proline-rich protein 2H	7.9
	<i>Spr2i</i>	Small proline-rich protein 2I	6.8

¹also cornified envelope cross-linking
FC: Fold Change

Predominant biological processes according to the list of the 2577 up-regulated genes

Validation by qRT-PCR

Up-regulation of antimicrobial defense and protease/protease inhibitor genes

3- Gene expression signature in the skin from PSD patients

Immunofluorescence staining

(KLK : kallikrein ; CSTA : cystatin A = Stfa in mouse; white dotted line: dermo-epidermal junction)

Western-blot

(Protein extracts from total skin)

Up-regulation of KLK7, KLK13 and CSTA in the skin from PSD patients

CONCLUSION

Genes strongly up-regulated in *Cdsn*^{EP-/-} E18.5 embryos only

Cxcl5, *Lce3d*, *Il1b*, *Mmp3*, *Cxcl3*, *Serpinb6d*, *Ccl20*, *Slpi*, *Ptgs2*, *Il6*, *Ifi202b*, *Gprc5a*, *Lif*, *Arc*, *Ifnk*, *Xrcc2*, *Ereg*, *Fosl1*, *Ngf*

Genes strongly up-regulated in both *Cdsn*^{EP-/-} E18.5 embryos and *Cdsn*^{EP-/-} mice

Il24, *Epgn*, *Areg*, *Spr2g*, *Spr2e*, *Spr2i*, *Lce3f*

Genes strongly up-regulated in *Cdsn*^{EP-/-} mice only

S100a9, *Spr2d*, *Mup1*, *S100a8*, *Spr2a2*, *Il1f6*, *Defb4*, *Rptn*, *Cxcr2*, *Lcn2*, *Spr2h*, *Saa1*, *Stfa3*, *Slc7a11*, *Fgg*, *Tpsab1*, *Nppb*, *Ly6g*, *Klk13*, *Mup10*, *Gsdmc*, *Stfa1*, *Dhrs9*, *Adam8*, *Mup13*, *Stfa2*, *Mup2*, *Spr2a2*, *Mup7*

- Skin barrier repair response:** cornification genes shared between the two *Cdsn*-deficient mice models.

- Unsuspected **induction of genes coding KLK13 and Stfa/CSTA** in “chronic” PSD mouse model and patients.

- Functional significance of KLK13 and CSTA in PSD?**

Our transcriptomic analysis reveals gene signatures biologically relevant for Peeling Skin Disease