DEBRA Members’ Weekend 2017

EB therapy research in Dundee: an update

Knocking out the faulty gene & Skipping the faulty exon

Peter van den Akker
EB therapy research in Dundee: an update

Knocking out the faulty gene
&
Skipping the faulty exon

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What am I going to tell you?

- Recap of my presentation of last year
  - What does **the skin** look like?
  - What is **wrong** in EB?

- EB therapy research in Dundee: where do we stand?
  - Gene knock-out for EBS
  - Exon-skipping for RDEB
The skin – A wall - Protection

Epidermis

Dermis

Hadrian’s Wall
Epidermolysis bullosa simplex

Basal skin cells

Epidermis

BMZ

skin cells (keratinocytes)

Basal cells

Dermis

Hadrian’s Wall
Basal keratinocytes
Keratin: support to cells

Basal skin cells (keratinocytes)

BMZ
Keratin 5&14

Stretch

BMZ
Keratin 5&14

Basal skin cells (keratinocytes)
EBS
Keratin cannot be made well

BMZ Keratin 5&14

Basal skin cells (keratinocytes)

Stretch

BLISTER

BMZ Keratin 5&14

Basal skin cells (keratinocytes)
KNOCKING OUT THE FAULTY GENE

EBS Therapy Project in Dundee
In a normal skin cell

DNA
KRT14

RNA copy
KRT14

Protein
Keratin 14

[Image of cell with green and blue fluorescence]
In an EBS skin cell

DNA
KRT14

RNA copy
KRT14

Protein
Keratin 14

Disease-causing Mutation

Mutation
**KRT14 gene knock-out**

DNA KRT14 → RNA copy KRT14 → Protein Keratin 14

Mutation

Disease-causing Mutation

Mutation-specific antisense oligonucleotides (ASOs) ("RNA scissors")
**KRT14 gene knock-out**

DNA
KRT14

RNA copy
KRT14

Protein
Keratin 14

Mutation
No match!

Disease-causing Mutation

Mutation-specific antisense oligonucleotides (ASOs) ("RNA scissors")
**KRT14 gene knock-out**

- **DNA**
  - KRT14

- **RNA copy**
  - KRT14

- **Protein**
  - Keratin 14

Mutation-specific antisense oligonucleotides (ASOs) ("RNA scissors")
**KRT14 gene knock-out**

Disadvantage: mutations specific $\rightarrow$ suitable only for a limited number of people with EBS
Benign DNA variations (SNP)

DNA KRT14  RNA copy KRT14  Protein Keratin 14

Everybody carries these benign variations (also people with EBS)
Targeting benign SNPs

DNA  RNA copy  Protein
KRT14  KRT14  Keratin 14

Same result as targeting a mutation
Targeting benign SNPs

DNA KRT14   RNA copy KRT14   Protein Keratin 14

Same result as targeting a mutation (but does not make a difference for the protein)
Idea: target the benign DNA variations (SNPs) that are linked to the mutations

DNA
KRT14

RNA copy
KRT14

Protein
Keratin 14

\[ \text{DNA} \quad \text{RNA copy} \quad \text{Protein} \]

- Disease-causing Mutation
- Benign SNP

Match!

Mutation
SNP
Idea: target the benign DNA variations (SNPs) that are linked to the mutations

DNA
KRT14

RNA copy
KRT14

Protein
Keratin 14

C
G

Disease-causing Mutation

Benign SNP

Mutation

SNP
Targeting many different mutations!

DNA  
KRT14

RNA copy  
KRT14

Protein  
Keratin 14

![Diagram of DNA, RNA copy, and protein with SNPs and mutations](image-url)
Targeting many different mutations!

Advantage: the strategy can be used to target many mutations and treat many patients
NOW WHERE DO WE STAND?
Treating cultured skin cells with ASOs
Treating skin with ASOs
KRT14 knockdown: summary

- Works in cultured skin cells
- Does not work as well in skin (skin explant system) as in cell culture
- We are trying to find out why this is, in order to improve the knockdown in our skin explant system and make it applicable for people with EBS
RDEB Therapy Project in Dundee

SKIPPING THE FAULTY EXON
Dystrophic epidermolysis bullosa

Anchoring fibrils: attach epidermis to dermis

Basal skin cells (keratinocytes)

Anchoring fibrils
Composed of Type VII collagen

Epidermis

Dermis
Dystrophic epidermolysis bullosa
Anchoring fibrils are absent

Basal skin cells (keratinocytes)
Keratin 5&14
Anchoring fibril
Composed of Type VII collagen

Stretch

Epidermis
Dermis
BMZ
In normal skin

DNA COL7A1 → RNA copy COL7A1 → Protein Type VII collagen

Normal skin
Type VII collagen
Nuclei
In RDEB skin

DNA
COL7A1

RNA copy
COL7A1

Protein
Type VII collagen

RNA copy

DNA

COL7A1

RDEB

Type VII collagen absent
Nuclei
**COL7A1 exon skipping**

DNA

RNA copy

Protein

COL7A1

COL7A1

Type VII collagen
**COL7A1 exon skipping**

<table>
<thead>
<tr>
<th>DNA</th>
<th>RNA copy</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL7A1</td>
<td>COL7A1</td>
<td>Type VII collagen</td>
</tr>
</tbody>
</table>

[Diagram showing DNA, RNA copy, and protein for COL7A1 exon skipping]
Normal skin

RDEB
Goal of exon skipping

Normal skin

Shorter but functional protein

RDEB
NOW WHERE DO WE STAND?
Exon skipping for **COL7A1**
Cultured RDEB cells make new type VII collagen
Exon skipping for *COL7A1*
RDEB skin makes new type VII collagen
COL7A1 exon skipping: summary

- Works well for certain exons in cultured cells and skin grafts
- Does work less well for other exons
- We are trying to find out why this is
- We need to improve the exon skipping efficiency
- We are studying whether the skipped protein is truly functional
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Thank you for your attention

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