# Inherited Skin Fragility Syndromes

## Epidermolysis Bullosa Simplex (EBS)
- Autosomal dominant inheritance
- Basal keratins K5, K14
- Blister formation in basal layer
- Generalized form

## Epidermolytic Hyperkeratosis (EHK)
- Autosomal dominant inheritance
- Suprabasal keratins K1, K10
- Suprabasal blister formation
- Generalized and mosaic form
Hotspot Mutations Frequently Cause EBS and EHK

Keratin 14 (EBS) and Keratin 10 (EHK)
Mouse Models that Genetically Mimic EBS and EHK are Not Viable
An Inducible Mouse Model for EBS

K14 promoter Inducible Cre

Mutant K14 NEO

Mutant K14 LoxP LoxP

RU486

Focal activation of mutant K14
Inducible Mouse Models for EBS and EHK

EBS Blister Induction

10 days after blister induction

6 months after blister induction

EHK Blister Induction

6 months after blister induction (same mouse)
What lessons have we learned from these mouse models?
Inducible EHK Mouse Models
Mimic Mosaic Forms of EHK in Humans

Inducible EHK mouse
Control mouse
Inducible Mouse Models Reveal the Role of Stem Cells in Mosaic Skin Diseases

EBS
- Focal activation of mutant K14
- Induced EBS blisters
- Blister healed by migrating normal stem cells
- RU486

EHK
- Focal activation of mutant K10
- Induced EHK blisters
- Blisters do not heal due to persistence of defective stem cells
- RU486

Stem cell
- Stem cell with mutant K10
- Progeny of stem cell with mutant K10
Mice that Express the mt K14 Allele at ~50% of wt K14 have a Subclinical Phenotype

No Phenotype

<table>
<thead>
<tr>
<th>Threshold</th>
</tr>
</thead>
</table>

Phenotype

<table>
<thead>
<tr>
<th>wt K14</th>
<th>mt K14</th>
</tr>
</thead>
</table>

\[\text{wt K14} \quad \text{mt K14}\]
• Select for stem cells
• Introduce therapeutic agent via lentiviral vector, ..... 
• Increase ratio of wild type to mutant protein
  - Over-expression of wt protein
  - Reduction of mt mRNA via siRNA

EBS

↓

Select for genetically modified keratinocytes ?

➢ Selective advantage of non-phenotypic stem cells

EHK

↓

➢ No selective advantage of genetically modified stem cells
  ➢ Drug selection required
Grafting Chamber

upper chamber

mixture of keratinocytes and fibroblasts

to

host skin

lower chamber

1 week

graft

host skin

muscle fascia
Grafting Chamber on Nude Mouse
Normal Epidermal Stem Cells Have a Growth Advantage over EBS Stem Cells in a Graft Environment
Using the Mouse EBS Model to Test Gene Therapy Approaches *In Vivo*

- **mK14\text{neo}/K14-CrePR1**
- **mK14\text{loxP}**

- Stem cell enrichment
- Genetic modification
- Transplant, RU486
Can the EBS-DM phenotype be corrected by decreasing the expression of mtK14 (siRNA)?

Testing the Specificity of the mtK14 siRNA within Cells

mtK14 siRNA and EGFP-wt or mtK14 into Ptk2 cells (no K14)

- Fluorescent microscopy
- FACS
- qRT-PCR
Specificity of mtK14 siRNA within Cells

FACS Analysis: 1:20 ratio, 72h
Therapeutic Strategy for EBS

Lentiviral Vector

Therapeutic Gene/Construct

EBS-DM stem cells have a selective disadvantage in a graft environment
Can this approach be used to make mouse models for other inherited skin diseases?
Strategy for Generating a Partially Humanized Inducible Mouse Model for PC

A

B
Strategy for Generating a Completely Humanized Inducible Mouse Model for PC

A

B

C

D
Acknowledgements

Inducible Models for Inherited Skin Diseases

**EBS**
Tongyu Cao
Minsue Chen
Daniel Young
William Buitrago

**EHK**
Meral Arin
Jiang Chen

**PC**
Jiang Chen

**Funding**
NIAMS
NICHD
DebRA-America
DebRA-UK
F.I.R.S.T.
PC-Project