Sensing
human epidermal keratinocyte radiosensitivity
Human skin structure

- Epidermis
  - Cellule de Langerhans
  - Keratinocyte
  - Couche cornée
- Dermis
  - Mélanocyte
  - Couche basale
  - Glande sébacée
  - Glande sudorale
  - Follicule pileux
- Adipous tissue
  - Fibre nerveuse
  - Fibroblaste
  - Matrice extracellulaire
  - Capillaire sanguin
  - Adipocyte
Human epidermis: highly renewing tissue every 28 days
Specific functions and markers: granular layer

barrier function

Cathepsin D  Involucrin

Expression in culture
anti cathepsin D
day 3  day 10
Specific functions and markers: spinous layer

differentiation

Stathmin
Expression in culture
anti stathmin
d3   d10

Keratins
K1/K10

20µm
Specific functions and markers: basal layer

Proliferation
Self renewal

INTEGRINS

\[
\text{ITG } \alpha_6 \\
\text{ITG} \beta_1
\]

KERATINS

K5/K14

GAPDH

\[
\begin{align*}
\text{d3} & \quad \text{d10} \\
\text{Anti-GAPDH}
\end{align*}
\]
The fascinating basal layer!

**stem cell**
- dormant
- high growth potential
- asymmetric division

**progenitors**
- proliferating cell
- symmetric division

Terminal differentiation

basal layer
Skin carcinoma: one of the most frequent human cancer

- Papilloma benign
- Baso-cellular Carcinoma BCC
- Spino-cellular carcinoma SCC
Cancer proneness

Papilloma and SCC differentiated tumor differentiated cells spinous layer

BCC undifferentiated tumor tumor/cells basal layer
Skin carcinoma: one of the most frequent human cancer

S Sell, Critical Reviews in Oncology/Hematology (2004)
Human Epidermis
a very radiosensitive tissue

Early side-effects of radiotherapy: radiodermatitis
Frequent late effects in normal tissue

- Atrophy
- Necrosis

- Hyperplasia
- Fibrosis

- Keratosis
Skin carcinoma: a radiation-induced cancer

- Papilloma (benign)
- Baso-cellular Carcinoma (BCC)
- Spino-cellular Carcinoma (SCC)
Classical concepts on epidermis radiosensitivity and cancer proneness
Human epidermis intrinsic radiosensitivity

Living epidermis

- granular layer
- spinous layer
- basal layer

very radiosensitive
very radioresistant
radioresistant
Late effects in normal tissue

stem cell depletion?

Uncontrolled stem cell activation?

keratosis

X20

X20
Questions...

1. Radiosensitivity from differentiated keratinocytes to stem cells?

2. Key player genes?

3. Role of each cell type in skin radiation damage?
plastic surgery

Normal skin samples

Normal skin samples

tripsine

Primary keratinocytes

Skin sections
I. Intrinsic radiosensitivity of differentiated keratinocytes?
normal human keratinocytes

trypsin

tissue culture

calcium, confluency

differentiated keratinocytes

time course 3 to 72 hours
Two doses:

- **2 Gy**
  - dose per fraction / radiotherapy
  - Co\textsuperscript{60} sources, 280 mGy/min, 8 min

- **10 mGy**
  - very low dose, medical diagnostic
  - Co\textsuperscript{60} source, no shielding, 2.15 mGy/min, 4 min

Assays:
- toxicity
- gene profiling
- protein expression and activity
Immediate toxicity

Differentiated keratinocytes 2 Gy and 10 mGy:

- cell viability not affected at any time and dose over 3 days

- Differentiated keratinocytes radioresistant!

Mechanisms of this resistance?

Gene profiling
Response to 2 Gy

Genomic response within 3 hours

- Genes related to cell defence and cell activation
- Energy production (ATP)
- Decreased differentiation
- Re-entry in cell cycle

370 genes
6%

Genomic reprogramming

Lamartine, J Cell Biochem, 2005
Id2 protects keratinocytes

ID2 knock-down: radiosensitivity!

ID2 over-expression: radioresistance

Baghdoyan, J Biochem Cell, 2005
Any genomic response after 10 mGy?

Strong genomic response after 10 mGy in differentiated keratinocytes!
Specific time-related responses

2 Gy:  . inductions at 3 hours and 24 h
     . repressions at 48 h

10 mGy:  . repressions at 48 hours
         . inductions at 72 h

- black: repressed genes
- red: induced genes
Which genes?

- 200 specific 10 mGy genes
- 140 known cellular functions, 60 unknown

<table>
<thead>
<tr>
<th>Gene/protein</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATA-1</td>
<td>GATA binding protein 1</td>
</tr>
<tr>
<td>GATA-3</td>
<td>GATA binding protein 3</td>
</tr>
<tr>
<td>AP-2</td>
<td>Activator protein 2</td>
</tr>
<tr>
<td>Ik-1</td>
<td>Ikaros</td>
</tr>
<tr>
<td>IL-6 RE</td>
<td>LI6 RE binding protein</td>
</tr>
<tr>
<td>SP-1</td>
<td>SP1 transcription factor</td>
</tr>
<tr>
<td>SRY delta</td>
<td>serendipity</td>
</tr>
</tbody>
</table>

7 putative transcription factors orchestrating low dose response!

*Franco N et al. Radiation Research, 2005*
GATA3: orchestrates protection from low doses!

- **GATA3 binds to promoters of genes activated by 10 mGy**
  - Chromatin immuno-precipitation with anti-GATA3 et anti-GATA1 and PCR

- **GATA3 knock-down: increased toxicity to 10 mGy**

*Bonin et al., BMC Genomics, 2009*
Conclusion

- Differentiated keratinocytes are radioresistant

- Signatures of radiation exposure in differentiated keratinocytes:
  - 2 Gy: specific responses to clinical dose
  - 10 mGy: specific responses to diagnostic dose

Genes involved in radioresistance and cell activation, ID2

Low dose response orchestrators, GATA3
Radiation-induced reprogramming? 

- Stem cell 
- Early progenitors 
- Late progenitors 
- Differentiated keratinocyte 

- Squamous cell 
- Carcinoma 
- Papilloma 
- No tumour
Skin group, CEA, Evry

Jerôme Lamartine
Noréli Franco
Keratinopoiesis

Undifferentiated basal cells

Differentiated cells

dormant stem cell

cycling progenitors

LGRK
Questions...

1. Radiosensitivity of basal keratinocytes?

2. Key gene players?

3. Role of each cell type in skin radiation damage?
Cell isolation from normal skin using membrane markers

Differentiated 90%

α6 integrin
 tranferrin receptor

Cytometry

Progenitors 10%
Stem cells 0.1%

D cells
α6 dim

CD71 brij

α6 brij

TA cells

α6 Fluorescence

CD71 Fluorescence

Differentiated 90%
Radiosensitivity of basal keratinocytes?

Progenitors inta6+/CD71-
Stem cells Inta6+/CD71+

2 Gy, 10 mGy single dose γ rays
Immediate radiosensitivity

Toxicity of 2 Gy at 72 hours after exposure, XTT assay

Progenitors

Stem cells

0 Gy

2 Gy

MT Martin
Cell survival at 15 days after 2 Gy

![Cell survival images](image)

Colony Forming Efficiency

**progenitors**

**stem cells**

Sensitivity of progenitors and stem cells to radiation exposure.

*Colony Forming Efficiency*

- **Sensitive**
- **Resistant**

*p < 0.05*  

**Rachidi W et al., Radiat Oncol 2007**
Residual $\gamma$H2AX foci at 24 h

Distribution of foci per cells 24 h after 2 Gy

- only progenitors had $> 4$ foci/cell
- confirmation of their radiosensitivity
Progenitors sensitive to 10 mGy!

Short-term toxicity (test XTT)

<table>
<thead>
<tr>
<th>Time</th>
<th>0 Gy</th>
<th>10 mGy</th>
<th>2Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72h</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Clonal survival (colony assay)

<table>
<thead>
<tr>
<th>Dose</th>
<th>0 Gy</th>
<th>10 mGy</th>
<th>2Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>survi</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PE control : 10 3 %
Summary radiosensitivity

progenitors: radio-sensitive
stem cells: radio-resistant

Rachidi W et al., Radiother Oncol, 2007

One of the first publications
demonstrating SC radioresistance
for human cells (hMSC, Chen, 2006)
and the first for skin (mouse and human)

Harfouche et al., Mut Res, 2010
BM mesenchymal stem cells

- Apoptotic cell death rate: 11% after 9 Gy (annexin V)

- MSC: *in vitro* radioresistance

*Chen MF*, *I J Radiat Oncol Biol Phys*, 2006
Human skin radiation syndrome

. Progenitors radiosensitive: dry desquamation (from 5 Gy) due to massive cell death in the progenitor compartment

. stem cells more resistant higher doses required
12 à 20 Gy: moist desquamation, necrosis

Mechanisms of SC resistance?
Global DNA repair activated in SC – 2 Gy

Comet assay: single strand DNA breaks and base damage
DNA DSB repair activated in SC - 2 Gy

**Repair kinetics**

<table>
<thead>
<tr>
<th>Post-irradiation time</th>
<th>Foci per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>5min</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>15min</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>30min</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>4H</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>24H</td>
<td>10 ± 1</td>
</tr>
</tbody>
</table>

* denotes significant difference compared to control.

**Foci distribution at 24 H**

<table>
<thead>
<tr>
<th>Foci per cell</th>
<th>% of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>2</td>
<td>30%</td>
</tr>
<tr>
<td>3</td>
<td>15%</td>
</tr>
<tr>
<td>4</td>
<td>5%</td>
</tr>
<tr>
<td>5</td>
<td>10%</td>
</tr>
<tr>
<td>≥6</td>
<td>5%</td>
</tr>
</tbody>
</table>

Progenitors
50% of cells with residual foci = dead cells
DSB induction and repair in progenitors after 10 mGy

<table>
<thead>
<tr>
<th>Nb foyers/nucléus</th>
<th>0 Gy 5 mn</th>
<th>10 mGy 5 mn</th>
<th>0 Gy 24 hr</th>
<th>10 mGy 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp1</td>
<td>0.03</td>
<td>0.16</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Exp2</td>
<td>0.03</td>
<td>0.54</td>
<td>0.013</td>
<td>0.02</td>
</tr>
<tr>
<td>Exp3</td>
<td>0.05</td>
<td>0.32</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

↑ DSB (x5 to 10)

Complete repair at 24 hours

* p<0.05
Stem cells: rapid and efficient DNA repair, a mechanism of SC radioresistance!

Progenitors: sensitive, slow and defective repair

*Harfouche G et al., Stem Cells 2010*
Mouse hair follicle radiosensitivity?

Bulge SC markers: integrin α6+, CD34+

Bulge cells compared to all other keratinocytes, from hair follicle and epidermis, through CD34 antigen
DNA repair activated in mouse bulge stem cells

Sotiropoulou PA, Nature Cell Biol, 2010
One mechanism: higher NHEJ and DNA-PK activity in mSC

NHEJ estimated by repair of reporter plasmids (a and b)
Sotiropoulou PA, Nature Cell Biol, 2010
Hematopoietic stem cells: resistant!

intrinsic radiosensitivity

clonogenic survival

More rapid DNA repair in HSPC

Foci H2AXg

M Mohrin, Cell Stem Cell, 2010
Other mechanisms of resistance?
more active cell signalling upstream of DNA repair in SC?

Gene profiling after 2 Gy

progenitors 4700

stem 227

Genes specifically regulated in irradiated SC

MT Martin
The most significant network of up-regulated genes: **cytokines**
FGF2 pathway is activated in SC

FGF-R1 → HP2 → GRB2 → H-Ras → MERK1 → ERK1/2 → TFs

extracellular → cytoplasm → nucleus

Radioresistance? DNA repair?
Blocking FGF2 pathway inhibits DNA repair
Blocking FGF2: SSB repair
Blocking FGF2: DSB repair

- Inhibition du récepteur

- Inhibition des MapKinases
Result:

in stem cells

Autocrine FGF2 involved in activation of DNA double strand break repair

Harfouche G et al., Stem Cells, 2010
Modelling FGF2 in stem cells

Several FGF2 isoforms:
- nuclear 24 kD
- cytoplasmic 18 kD
- Secreted form 18 kD
Nuclear and secreted forms of FGF2 protect SC from radiations
Progenitors: exogenous FGF2 activates DNA repair

DNA damage γ-H2AX
FGF2 supplementation on progenitors

- Foci per cell
- Post-irradiation time
- 5min, 15min, 4H, 24H

- without FGF2
- FGF2 at 1H before 2 Gy
- FGF2 at 3H

* *
Clinical use of FGF2 ?

Already described as a prosurvival factor

*Tomlinson Cancer Res 2009*

Now a prorepair factor !
Proposition: a new way to protect human skin

2011: yes !
FGF2-P a new way to protect and repair human epidermis

*Zhang et al., IJROBP, 2011*
Laboratory of Genomics and Radiobiology of Keratinopoiesis
Many questions...and some answers

1. Radiosensitivity from differentiated keratinocytes to stem cells?

2. Key player genes?

3. Role of each cell type in skin radiation damage?
Human epidermis intrinsic radiosensitivity

Classical concepts

G: very radioresistant
S: radioresistant
B: very radiosensitive

CEA skin group

G: very radioresistant
S: radioresistant
P: very radiosensitive
SC: radioresistant
Cellular origin of tumors...still an opened question

- Stem cell
- Progenitors
- Differentiated keratinocyte
- Squamous cell carcinoma
- Papilloma
- No tumour
Normal tissue damage

. FGF2: radiosensitive patients could be protected
. FGF2 and repair/radiodermatitis
Evry Genopole
CEA laboratory 30 km south of Paris