# Regional variation in epidermal susceptibility to ultraviolet induced carcinogenesis reflects proliferative activity of epidermal progenitors

**Edwige Roy**, Zoltan Neufeld, Luca Cerone, Ho Yi Wong, Samantha Hodgson, Jean Livet, Kiarash Khosrotehrani

## Background and proposal

Epidermal carcinomas:
- the most common human malignancies
- linked to ultraviolet (UV) irradiation

**Key conditions** for a given cell **to form a cancer** are multiple:
i) to accumulate mutations that may randomly derail some key oncogene
ii) to survive long term and avoid entering terminal differentiation
iii) to have the capacity to generate a clone of similarly mutated cells that is expanded

All three conditions may be significantly affected by UV exposure.

Moreover, we and others have demonstrated that, in the IFE, **two modes of epidermal clonal progression coexist** according to anatomical regions. To date it is unknown whether epidermal clone size evolution observed during homeostasis is altered upon chronic UV exposure.

As such, it is essential to show how different clones of epidermal cells respond to UVB chronic exposure in order to understand the initial steps of carcinogenesis.

**Aims**

Aim 1: Evaluation clone size dynamics in chronically UVB irradiated skin
Aim 2: Ask if specific clones would have more propensity to form skin cancer either through accumulation of mutations or through their proliferative potential.

## Experimental approach

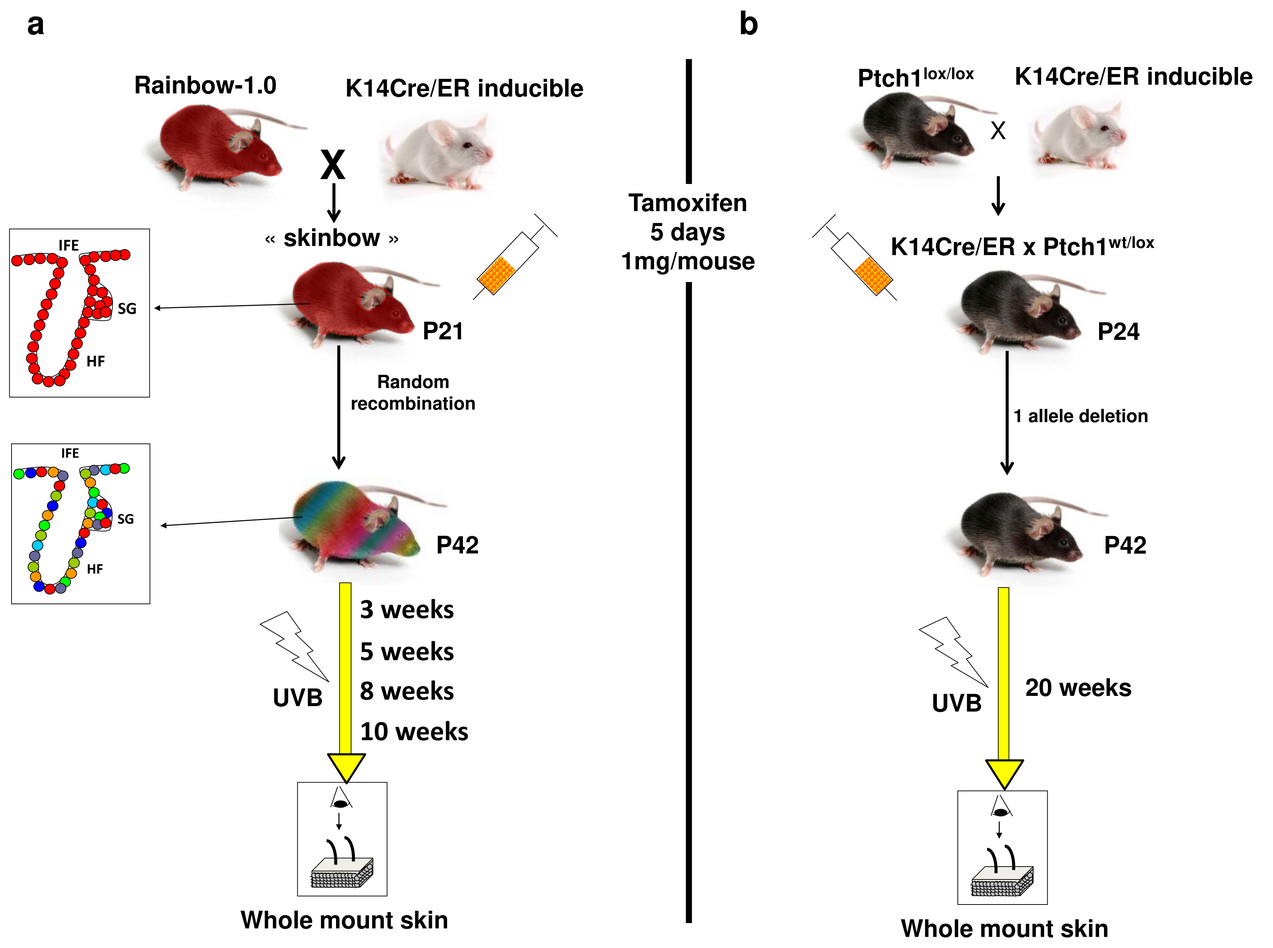



**Fig1 : a)** We used transgenic mice containing a Brainbow-1.0 cassette under the control of an ubiquitous promoter allowing the random expression of td-Tomato (red), Cerulean (blue) or eYFP (yellow) upon cre recombination. When crossed with K14-Cre/ERT2 transgenic mice, this allowed recombination in all basal keratinocytes regardless of their anatomical location and following their fate over time after tamoxifen injection at P21 (1mg/mouse/day for 5 consecutive days for high density). Back skin of mice from 6 weeks of age was exposed to UV-B radiations 3 times a week at a suberythemal dose. Then, the back skin of mice was harvested after 3, 5, 8 and 10 weeks of UV-B irradiation. Thus, wholemount samples were analyzed by confocal microscopy. The control groups were not exposed to the radiation. **b)** We crossed transgenic mice containing the *Ptch1 gene* flanked by 2 loxP sites and a *K14-Cre/ERT2* transgenic mice allowing recombination in all basal keratinocytes regardless of their anatomical location to track their fate over time. Tamoxifen injections performed at P24 (1mg/mouse/day for 5 consecutive days for high density) triggered recombination in keratin 14-expressing cells. The back skin of mice was exposed to UV-B radiations 3 times a week at a suberythemal dose. Then, the back skin of mice was harvested after 20 weeks of UV-B irradiation. Thus, wholemount samples were analyzed by confocal microscopy. The control groups were not exposed to the radiation.

## Results

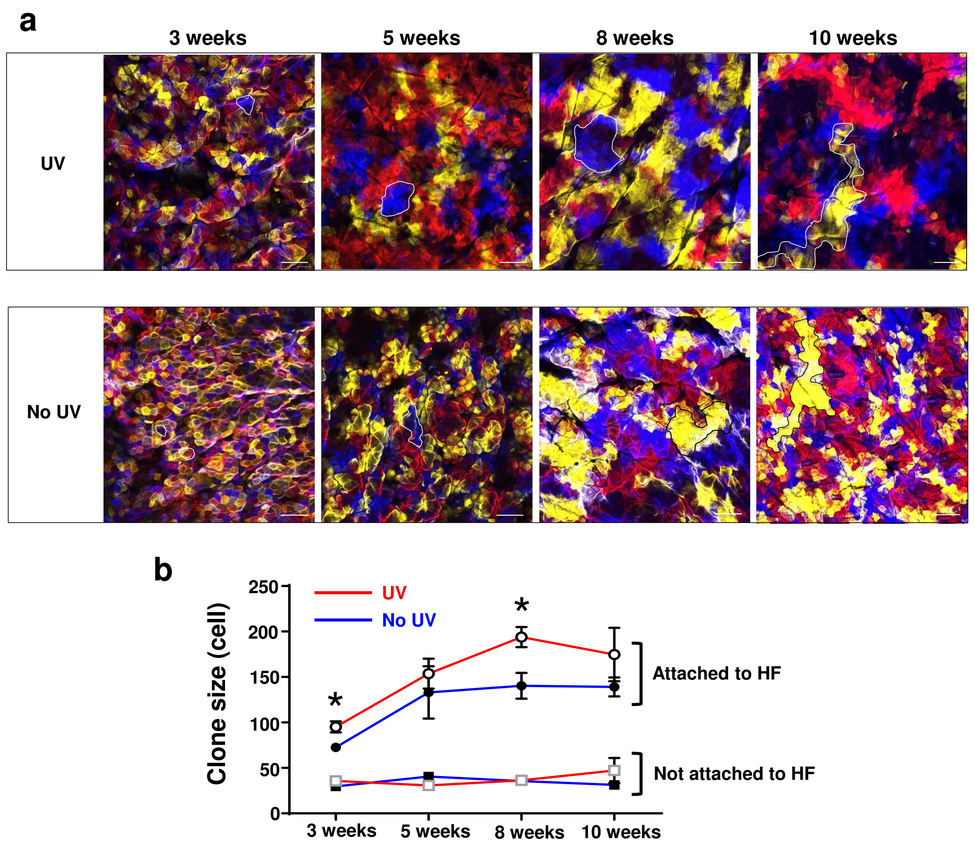



**Figure 2: IFE clones labeled at P21 continuously grow in size between 3 and 10 weeks upon UVB exposure and clones attached to hair follicle are larger and are more likely to be lager upon UVB irradiation.**
**a)** Photomicrograph represents a 2D optical section from a Z stack acquisition of whole mounted skin displaying IFE clones of different colours after 3, 5, 8 and 10 weeks upon chronic UVB exposure (up row) compared to unirradiated skin from control mice (bottom row) (bar = 100 µm).
**b)** Average clone size were plotted over time. Average size of clones attached to HF was significantly larger in the UVB-exposed group compared to the not UVB-exposed group. Mann-Whitney test, 3 weeks * p=0.026, 8 weeks * p=0.036. Data are represented as mean ± s.e.m. Of note, no difference was observed between UVB-exposed and not exposed clones distant from HF.

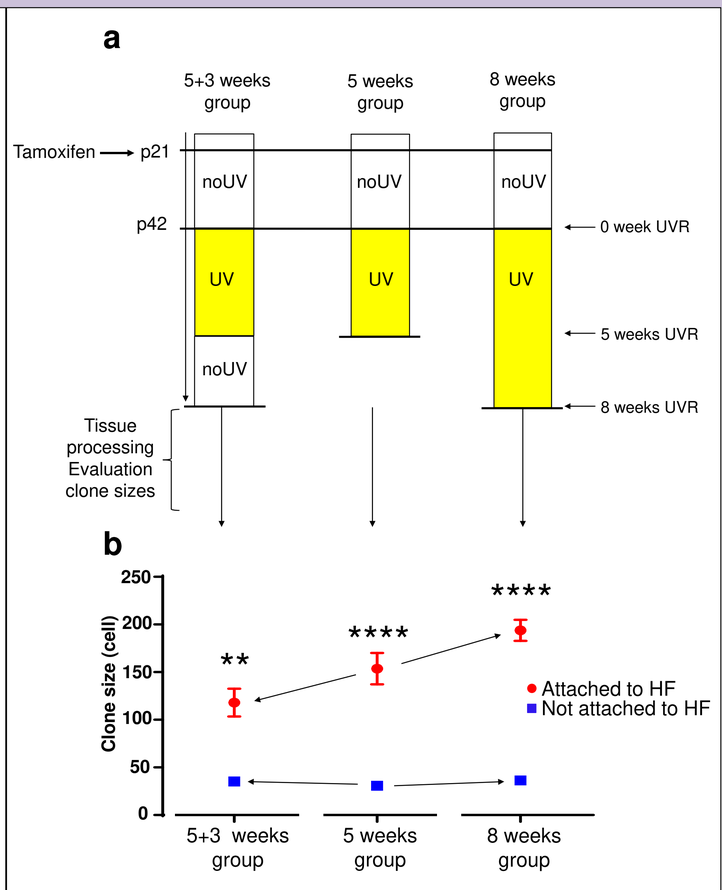



**Figure 3: UVB irradiation results in clone size increase that is rapidly returned to normal when UVB exposure is interrupted.**
**a)** Experimental design. Tamoxifen induced skinbow mice were exposed to UVR for 5 weeks before establishing 3 groups: a first group was sacrificed immediately (5 weeks group), a second group was UVB irradiated for 3 more weeks (8 weeks group). A third group was maintained 3 more weeks without UV irradiation (5+3 group).
**b)** Average clone size of attached (red circles) and non-attached (blue squares) clones were plotted regarding their group of UVB exposure. Mann Whitney, ** p=0.0043, **** p<0.0001. Data are represented as mean ± s.e.m.

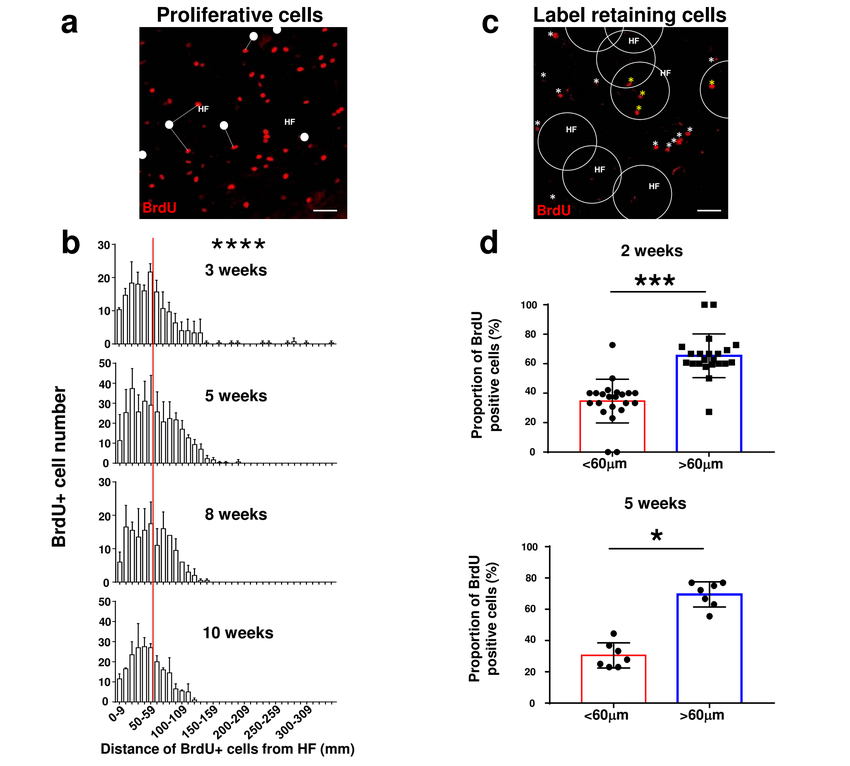
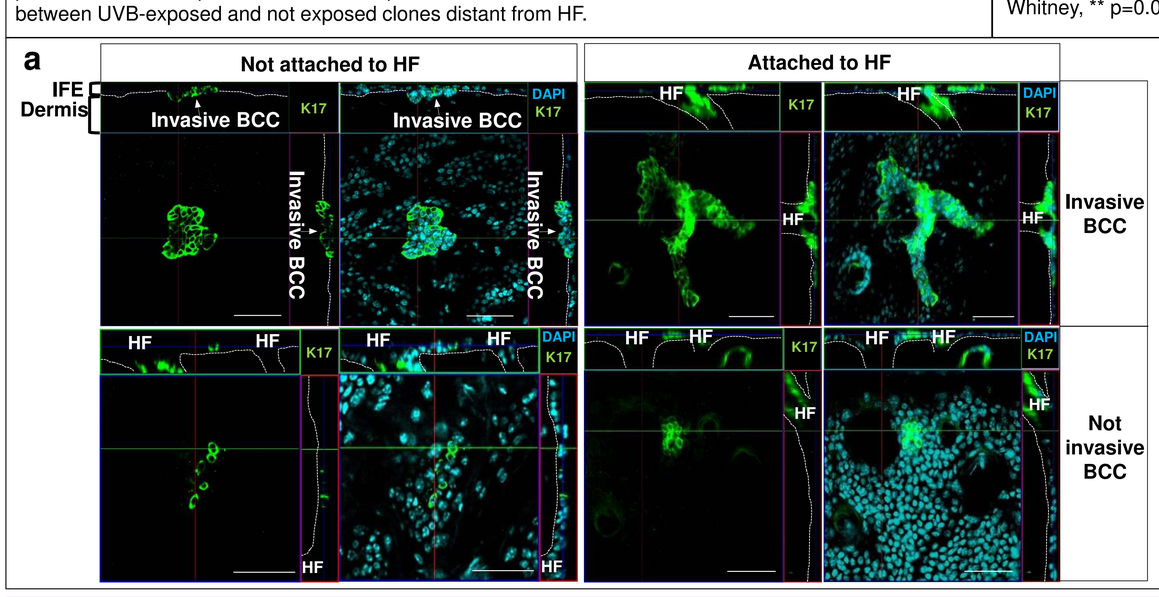



**Figure 4: Epidermal proliferative cells are mostly observed around hair follicles**
**a)** Photomicrograph represents a 2D optical section from a Z stack acquisition of whole mounted skin displaying BrdU+ proliferative cells (red). HF: hair follicle. White line: the shortest distance measured between a BrdU+ cell and the closest HF. (bar = 50 µm).
**b)** Bar charts showing distribution of the shortest distances between HF and BrdU+ proliferative cells at 3, 5, 8 and 10 weeks. The red line represent a cut off at 60 µm distant from HF. Data are represented as mean ± s.d.
**c)** Photomicrograph represents a 2D optical section from a Z stack acquisition of whole mounted skin displaying BrdU+ label retaining cells (red). White circles of 60 µm radius are centered on hair follicles representing the limit between adjacent and distant regions around HF. (scale bar=50um).
**d)** Bar charts showing the proportion of BrdU+ label retaining cells in the adjacent region to HF (less than 60um) compare to the distant region to HF (more than 60 um). Most of the BrdU+ cells are observed in the distant region. 2 weeks: *** p=0.0002; 5 weeks: * p= 0.0156. Data are represented as mean ± s.d.



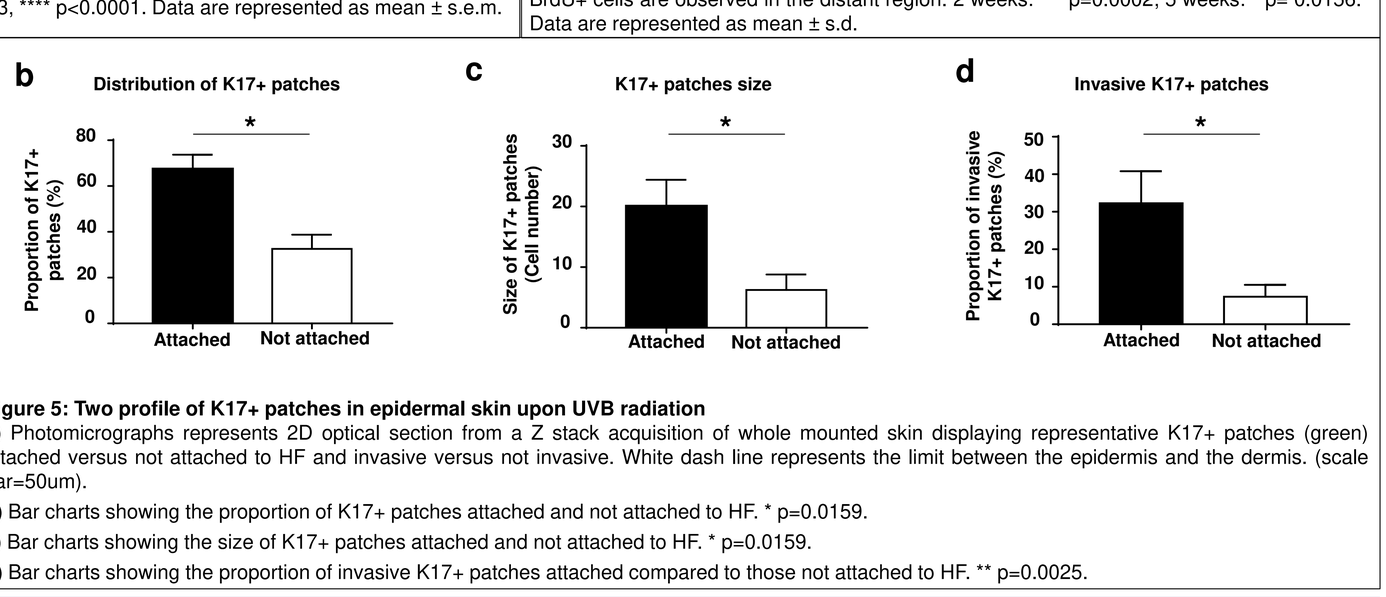




**Figure 5: Two profile of K17+ patches in epidermal skin upon UVB radiation**
**a)** Photomicrographs represents 2D optical section from a Z stack acquisition of whole mounted skin displaying representative K17+ patches (green) attached versus not attached to HF and invasive versus not invasive. White dash line represents the limit between the epidermis and the dermis. (scale bar=50um).
**b)** Bar charts showing the proportion of K17+ patches attached and not attached to HF. * p=0.0159.
**c)** Bar charts showing the size of K17+ patches attached and not attached to HF. * p=0.0159.
**d)** Bar charts showing the proportion of invasive K17+ patches attached compared to those not attached to HF. ** p=0.0025.

## Conclusion

Our findings suggest that, despite globally increased proliferation, **two modes of clone progression co-exist** and are **restricted to specific IFE regions**.
UVB induced proliferation was more focused around hair follicles resulting in cycling cells, larger clones and less label retaining as a result of **cyclin D1 overexpression**.
Although this does not seem to influence mutation accumulation, it **strongly affected hedgehog induced carcinogenesis**.

These findings have far reaching implications in our **understanding of the cell of origin of keratinocyte cancers and its relevance to the clinic**. It also highlights the possibility of **reducing skin cancer incidence** by altering the expression or activity of cyclin D1 in proximity of hair follicles.