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Interfollicular Epidermal Homeostasis: A Response to Ghadially, '25 Years of Epidermal Stem Cell Research'

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TO THE EDITOR

We would like to respond to the comments in the otherwise excellent review ''25 Years of Epidermal Stem Cell Research'', which highlight attempts to rebut the ''single progenitor'' (SP) model of epidermal homeostasis (Ghadially, 2011). As the article summarizes, the SP model derives from large-scale *in vivo* lineage tracing following the fate of a representative sample of proliferating keratinocytes over 1-year time courses. The results reveal that normal murine epidermis is maintained by a single population of cells that divide asymmetrically and symmetrically to generate equal numbers of progenitor and differentiating daughters (Clayton *et al.*, 2007; Klein *et al.*, 2007; Doupe *et al.*, 2010). The self-sustaining progenitor population explains how homeostasis is achieved without the need for continual stem cell proliferation (Jones *et al.*, 2007; Jones and Simons, 2008). It is important to stress that the SP model, derived from observation of proliferating cell behavior, does not conflict with the existence of quiescent stem cells, which have the potential to regenerate the epidermis following injury and/or generate colonies in culture (Clayton *et al.*, 2007). The available evidence is consistent with the epidermis containing both progenitors and stem cells, the former maintaining the interfollicular epidermis and the latter being mobilized following injury (Ito *et al.*, 2005; Levy *et al.*, 2005).

We were concerned to read that a ''highlight'' of 2011 was ''a rebuttal of the SP theory by multiple stem cell researchers'', based on a single review article in which the two authors present no new data, drawing on a selective reading of older literature to argue that two populations, stem cells and transit amplifying cells, are required for epidermal homeostasis (Kaur and Potten, 2011). The results said to be in conflict with the SP model are ''continuous labeling studies, cell cluster analysis, and clonogenic studies following irradiation''. In fact, none of these refutes the SP paradigm. Continuous labeling studies are non-discriminatory between different models of homeostasis, whereas cell cluster analysis gives a poor fit to the classical epidermal proliferative unit (EPU) model but is consistent with SP behavior (Potten and Major, 1980; Loeffler *et al.*, 1987; Klein *et al.*, 2008). The regeneration of the epidermis after irradiation is outside the scope of the SP model, which derives from the observation of homeostatic epidermis. In contrast to these proxy assays, lineage tracing reveals the behavior of proliferating cells directly, providing robust evidence for the SP model (Snippert and Clevers, 2011; Doupe and Jones, 2012).

A strength of the SP paradigm is that it is quantitative, embodied in a simple equation that enables it to be tested against data from thousands of individual epidermal cell clones. It is suggested that ''the observed spectrum of KC could result from a continuum of proliferation ability'' (Ghadially, 2011). The available data enable epidermal researchers, in collaboration with biophysicists or mathematicians, to define the parameters within this or any other proposed model and test it against published results. Unfortunately, this has not been done by those who argue for the ''continuum model'', which would appear to be at odds with the observation that epidermal clone size scales with time at late time points (Clayton *et al.*, 2007; Kaur and Potten, 2011).

It is further commented that ''a lack of adequate methods'' may have resulted in an inability to identify ''transit amplifying cells''. Given the demonstration in lineage-tracing experiments that labeled cells are representative of unlabeled epidermis out to a year, this seems unlikely (Clayton *et al.*, 2007; Doupe *et al.*, 2010). Further, although rare subpopulations of proliferating cells with different behavior may not be detected by clonal analysis, this could not be said to apply to transit amplifying cells, which have been proposed to represent most dividing cells in the basal layer (Potten, 1975).

Finally, it is stated that our lineage-tracing studies were only conducted on the tail epidermis. This is incorrect, as a quantitative analysis of the more typical epidermis of the mouse ear demonstrating that this also follows the SP model has been reported (Doupe *et al.*, 2010). This work used extensive three-dimensional imaging to show that EPU, as originally defined (regularly sized clonal units supported by a single stem cell and associated transit amplifying cells), do not exist in the ear epidermis (Potten, 1975). None of the clones at the 1-year time point conformed to the boundaries of the supposed EPU.

We hope that these remarks clarify confusion over the SP model. It is certainly the case that there is an urgent need for additional large-scale lineage-tracing studies, particularly post injury, to further resolve cell behavior during epidermal regeneration.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Abbreviations: EPU, epidermal proliferative unit; SP, single progenitor

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Vitamin D Receptor Mediates DNA Repair and Is UV Inducible in Intact Epidermis but Not in Cultured Keratinocytes

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TO THE EDITOR

Although providing a powerful approach for studying epidermal biology, cultured keratinocytes may imperfectly model a three-dimensional epidermis in which cells are architecturally ordered. We report two important examples of the limitations of cultured keratinocytes in understanding vitamin D receptor (VDR) photobiology in murine skin. Recently, the vitamin D signaling pathway has been implicated in skin cancer prevention through its role in cellular responses to UVB radiation–induced DNA damage, and demonstrations that *VDR*$^{-/-}$ mice are susceptible to UVB-induced epidermal tumors (Ellison *et al.*, 2008; Quigley *et al.*, 2009; Mason *et al.*, 2010; Teichert *et al.*, 2011). VDRs transactivation of certain genes is also mediated by a subunit of the nucleotide excision repair (NER)/transcription factor, TFIIH (Drané *et al.*, 2004), further suggesting a potential interaction between VDR and DNA repair.

We examined the dependence of NER on VDR in detail in several model systems. First, wild-type and *VDR*$^{-/-}$ mice (Teichert *et al.*, 2011) were irradiated with UVB, and removal of the most common UVB photoproduct, the cyclobutane pyrimidine dimer (CPD), was monitored by immunofluorescence. At 1 hour post UVB, both wild-type and *VDR*$^{-/-}$ mice exhibited significant CPD levels in epidermal keratinocytes (Figure 1a). In wild-type epidermis, CPDs were markedly diminished by 24 hours and undetectable by 48 hours post UVB. In contrast, in the *VDR*$^{-/-}$ epidermis, CPDs persisted at 24 hours, and were still clearly detectable at 48 hours, indicating impaired NER. CPD quantification indicated that even as early as 1 h post UV, the wild-type epidermis had fewer CPDs than the VDR$^{-/-}$ epidermis (Figure 1b).

To facilitate quantitative analysis, we also explored the role of VDR in DNA repair *in vitro*. Keratinocytes cultured from mice bearing floxed *VDR* and expressing cre recombinase did not significantly express *VDR* relative to control cells (Figure 1c and d). UVB-irradiated cells were assayed for CPDs and the pyrimidine(6,4)pyrimidone photoproducts (6–4PPs) using a standard immunoblot assay (Yeh and Oh, 2002). *In vitro*, where it was possible to harvest cells within seconds following irradiation, no differences in initial CPD or 6–4PP levels were discernible between wild-type and *VDR*-negative keratinocytes (Figure 1e), and both cell types were completely deficient in global genomic NER of CPDs over 48 hours, although equally proficient in repair of 6–4PP (Figure 1f and g). These results agree with previous observations that cultured rodent cells possess poor global genomic NER of CPDs (Tang *et al.*, 2000).

We then studied explanted epidermal sheets that better preserve skin architecture than do cultured cells while providing a more easily manipulable model system for quantitatively assessing VDR effects than whole animals. Following harvest (Teichert *et al.*,

Abbreviations: CPD, cyclobutane pyrimidine dimer; NER, nucleotide excision repair; VDR, vitamin D receptor