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Vitamin D receptor mediates DNA repair and is UV-inducible in intact epidermis but not cultured keratinocytes

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To the Editor

While 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 ultraviolet B radiation (UVB)-induced DNA damage, and demonstrations that *VDR*^{-/-} mice are susceptible to UVB-induced epidermal tumors (Ellison *et al.*, 2008; Mason *et al.*, 2010; Quigley *et al.*, 2009; Teichert *et al.*, 2011). VDR's 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. 1 hour post-UVB, both wild-type and $VDR^{-/-}$ mice exhibited significant CPD levels in epidermal keratinocytes (Fig. 1a). In wild-type epidermis, CPDs were markedly diminished by 24 hours and undetectable by 48 hours post-UVB. In contrast, in $VDR^{-/-}$ 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 hour post-UV wild-type epidermis had fewer CPDs than VDR^{-/-} epidermis (Fig. 1b).

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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 (Fig. 1c,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 (Fig. 1e), and both cell types were completely deficient in global genomic NER of CPDs over 48 hours, though equally proficient in repair of 6–4PP (Fig. 1f,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.*, 2011), explants from mice bearing floxed *VDR* and cre were irradiated through the stratum corneum and incubated for 46 hours before measurement of photoproducts by immunoblot assay. Floxed VDR explants expressing cre were significantly deficient in both CPD and 6–4PP repair (Fig. 1h,i). Hydroxyurea treatment did not significantly affect CPD levels at 46 hours, indicating that the observed CPD loss reflected true repair rather than dilution of DNA damage through replication, consistent with a lack of PCNA staining in explants (data not shown).

VDR has also been reported to be UVB inducible *in vivo* (Hong *et al.*, 2008; Lesiak *et al.*, 2011; Mallbris *et al.*, 2005), while others have reported that VDR is down regulated by UVB *in vitro* (Courtois *et al.*, 1998). We studied this difference in behavior in our mouse model systems. Irradiation of wild-type mice induced epidermal *VDR* mRNA levels 2–3.5-fold by 24 hours (Fig. 2a). In epidermal explants derived from wild-type mice, UVB induced *VDR* mRNA >6-fold by 24 hours, and sustained that level over 48 hours (Fig. 2b). As anticipated, *VDR*-negative explants exhibited undetectable *VDR* expression. These results indicate that UVB strongly induces *VDR* expression, consistent with *in vivo* mouse and human data. In contrast, cultured mouse keratinocytes only weakly induced VDR mRNA expression following UVB (Fig. 2c). Consistent with prior results from cultured human keratinocytes, VDR protein levels increased only slightly at low UVB doses and then actually appeared to decrease at moderate doses, though the observed differences at all UVB doses were not statistically significant (Fig. 2d).

These results confirm that *VDR* is strongly induced by UVB in intact epidermis, consistent with its role in promoting NER (Ellison *et al.*, 2008). These activities, however, are not reflected in cultured keratinocytes monolayers. Similarly, sonic hedgehog is expressed and repressed by vitamin D in epidermal explants but not in cultured keratinocytes (Teichert *et al.*, 2011). Cultured rodent cells have been commonly regarded as being defective in CPD repair; this phenotype has been ascribed to rodent cells' inability to express *DDB2* which encodes a DNA damage recognition protein (Hanawalt, 2001; Tan and Chu, 2002; Tang *et al.*, 2000). However, qualitative studies of DNA repair in intact epidermis typically demonstrate that repair of epidermal CPDs appears to proceed efficiently (Fig. 1 and e.g.,

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ref. (Mitchell *et al.*, 1990)), suggesting that *DDB2* expression is actually not limiting in intact mouse epidermis. It has also been reported that cultured mouse keratinocytes but not fibroblasts are actually repair proficient and express DDB2 (Pines *et al.*, 2009). Interestingly, this prior study utilized keratinocytes grown on a layer of fibronectin and collagen, and it is possible that extracellular matrix or intercellular interactions or another as yet undefined tissue-related factor may specifically modulate NER activity in epidermal keratinocytes.

In summary, *VDR* is a UVB-inducible gene that critically supports NER activity in intact murine epidermis, but these activities are poorly recapitulated in keratinocytes cultured from the same animals. The use of epidermal explants may represent an approach that preserves the biological behavior of epidermis while providing a facile substrate for detailed molecular studies more commonly associated with cultured cells.

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Figure 1. VDR supports NER in intact epidermis but not cultured keratinocytes

(a,b) After 4000 J/m² UVB, 2 day old wild-type (C57BL/6) and V*DR*^{-/-} mouse skin was harvested and stained at varying times with primary anti-CPD antibodies (1:1000, Cosmo Biosciences) and fluorescein-labeled secondary antibodies (a). Mean anti-CPD signal 1 hour post-UV (b). C,D) *VDR* expression in floxed *VDR* keratinocytes expressing cre recombinase (cre) or luciferase (con) under K14 promoter control assayed by RT-PCR normalized to *L19* expression (c) or Western blotting (d) (Teichert *et al.*, 2011). (eg) Floxed *VDR* keratinocytes expressing cre or control cells were irradiated with 177 J/m² UVB, and assayed immediately (e) or over time for CPDs (e,f) and 6–4PPs (e,g). (h,i) Epidermal explants from² day old mice with floxed *VDR* expressed control luciferase (con) or cre under K14 control. Following 354 J/m² UVB, explants were assayed for CPDs (h) and 6–4PPs (i) at 0 or 46 hours. A subset of control (con+HU) and VDR knockout (cre+HU) explants were treated with hydroxyurea before and following UVB (h). Higher UVB dose for explants were needed to generate CPD levels comparable to those in cultured cells, likely due to scattering/ reflection in whole epidermis.

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Figure 2. UV induction of VDR in intact epidermis but not cultured keratinocytes

(a) Wild-type mice were irradiated with increasing UVB doses, and epidermis was harvested at 24 hours for *VDR* mRNA levels by RT-PCR, normalized to *L19* expression. (b) Epidermal explants from wild-type (WT, \bullet) and *VDR*^{-/-} (\blacksquare) mice were irradiated with 354 J/m² UVB, and assayed at 0, 24 and 48 hours for *VDR* mRNA levels, normalized to *GAPDH* expression. (c,d) Cultured keratinocytes from floxed-*VDR* mice expressing control (\bullet) or cre (\blacksquare) were irradiated with increasing UVB doses, and assayed for *VDR* expression by RT-PCR, normalized to *GAPDH* expression (C) and by Western blotting, with results quantified and normalized to actin (d).