Journal of Steroid Biochemistry & Molecular Biology 121 (2010) 314–316

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

The vitamin D receptor, the skin and stem cells☆

Hilary F. Luderer, Marie B. Demay*

Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, 50 Blossom St, Thier 11, Boston, MA 02114, USA

ARTICLE INFO

Article history:
Received 9 November 2009
Accepted 30 January 2010

Keywords:
Nuclear receptor
Hair follicle
Stem cell
Keratinocyte
Anagen

ABSTRACT

The active metabolite of vitamin D, 1,25-dihydroxyvitamin D, has been shown to have pro-differentiation and antiproliferative effects on keratinocytes that are mediated by interactions with its nuclear receptor. Other cutaneous actions of the vitamin D receptor have been brought to light by the cutaneous phenotype of humans and mice with non-functional vitamin D receptors. Although mice lacking functional vitamin D receptors develop a normal first coat of hair, they exhibit impaired cyclic regeneration of hair follicles that leads to the development of alopecia. Normal hair cycling involves reciprocal interactions between the dermal papilla and the epidermal keratinocyte. Studies in mice with targeted ablation of the vitamin D receptor demonstrate that the abnormality in the hair cycle is due to a defect in the keratinocyte component of the hair follicle. Furthermore, expression of mutant vitamin D receptor transgenes in the keratinocytes of vitamin D receptor knockout mice demonstrates that the effects of the receptor that maintain hair follicle homeostasis are ligand-independent. Absence of a functional vitamin D receptor leads to impaired function of keratinocyte stem cells, both *in vivo* and *in vitro*. This is manifested by impaired cyclic regeneration of the hair follicle, a decrease in bulge keratinocyte stem cells with ageing and an abnormality in lineage progression of these cells, leading to their preferential differentiation into sebocytes.

1. Introduction

Vitamin D and its receptor have been shown to play an important role in epidermal cells [1]. Interest in the effects of the vitamin D endocrine system on the skin is based, in part, on the striking cutaneous phenotype of humans and animals with vitamin D receptor mutations [2–6]. In addition, the skin is the only known organ system capable of synthesizing all the components required for ligand-dependent transactivation by the vitamin D receptor: vitamin D can be synthesized in response to UV radiation, the enzymes required for 25 hydroxylation and 1 alpha hydroxylation are expressed in the skin as is the vitamin D receptor [7].

Initial studies directed at identifying the abnormalities responsible for the alopecia observed in humans and mice with mutant vitamin D receptors, focused on epidermal keratinocytes. Keratinocytes isolated from neonatal vitamin D receptor null mice are resistant to the antiproliferative and pro-differentiation effects of 1,25-dihydroxyvitamin D. However, these cells respond in an identical fashion to wildtype keratinocytes when calcium is used as an antiproliferative and pro-differentiation agent [8]. In contrast, *in vivo* studies demonstrate impaired acquisition of markers of keratinocyte differentiation in vitamin D receptor null mice between birth and 3 weeks of age [9]. These data suggest that the keratinocytes of the vitamin D receptor null mice are normal at birth but there is an age-dependent impairment in epidermal differentiation that precedes the development of alopecia.

The hair follicle is an organ that is composed of an epidermal and a dermal component. Hair follicle morphogenesis begins day 14.5 of gestation in the mouse embryo. Reciprocal interactions between the epidermal placode and the dermal condensate result in invagination of the epidermis which subsequently develops into a hair follicle [10]. This morphogenic phase of hair follicle development continues until 2 weeks postnatally with the formation of a hair shaft. At this stage of development, the hair follicles of the vitamin D receptor null mice are indistinguishable from those of their wildtype littermates. After the morphogenic period, the hair follicle undergoes cyclic regeneration with distinct phases: hair growth during anagen, apoptosis of the lower part of the hair follicle during catagen and rest during telogen. During this last phase, the dermal papilla component of the hair cycle is brought into proximity to a region of the hair follicle known as the bulge. The bulge, which develops into a distinct morphological entity the third week of life, resides below the sebaceous gland, and is thought to contain multipotent stem cells [11]. Reciprocal communications between the dermal papilla and the bulge result in initiation of a new anagen phase of the hair cycle.

☆ Special issue selected article from the 14th Vitamin D Workshop held at Brugge, Belgium on October 4–8, 2009.

* Corresponding author. Tel.: +1 617 726 3966; fax: +1 617 726 7543.
E-mail address: demay@helix.mgh.harvard.edu (M.B. Demay).

0960-0760/$ – see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.jsbmb.2010.01.015

2. Alopecia in VDR null mice

The first clinical evidence of the hair defect in the vitamin D receptor null mice is observed at approximately 4 weeks of age, the time of the first post-morphogenic anagen phase of the hair cycle. *In vivo* studies in the vitamin D receptor null mice demonstrated an inability to respond to an anagen initiating stimulus after the end of the morphogenic period [8]. These investigations demonstrated that the alopecia in the absence of a functional vitamin D receptor was due to a defect in cyclic regeneration of the hair follicle.

Studies were undertaken to identify whether the vitamin D receptor was required in the epidermal component of the hair follicle or in the dermal papilla cells. Hair reconstitution assays demonstrated that vitamin D receptor expression in the dermal papilla is not essential, but expression in the keratinocytes is [12]. Additional studies in transgenic mice demonstrated that expression of the vitamin D receptor in the keratinocytes of vitamin D receptor null mice was able to restore functional post-morphogenic hair cycles and to prevent the development of alopecia [13]. To dissect the regions of the vitamin D receptor required for these effects, mice with keratinocyte-specific expression of receptors that abolished ligand binding and nuclear co-activator recruitment were engineered. These studies demonstrated that the ability to bind ligand was not required for the vitamin D receptor to promote cyclic regeneration of the hair follicle [14]. Inability to recruit co-activators resulted in a mild phenotype. Thus, these investigations demonstrated that vitamin D receptor expression in keratinocytes was essential for post-morphogenic hair cycling and that these actions were ligand-independent.

3. The VDR and keratinocyte stem cells

The specialized bulge region of the hair follicle represents a niche of stem cells that are called into play to regenerate the lower portion of the hair follicle during anagen, but also give rise to sebaceous cells and contribute to cutaneous wound repair. Histological analyses of the skin of the vitamin D receptor null mice demonstrated expansion of the lipid-laden sebocyte compartment and formation of large lipid-laden dermal cysts with age. This raised the question as to whether the actions of the vitamin D receptor were to maintain the keratinocyte stem cell niche and/or play a role in lineage determination of these cells.

Similar to other stem cells, when plated at low density keratinocyte stem cells grow slowly and after 4 weeks in culture on feeder layers give rise to large colonies [15,16]. While the number of large colonies formed by cells isolated in the neonatal period was unaltered by absence of a functional vitamin D receptor, when cells were isolated from 4-week-old mice, colony formation was dramatically impaired in the vitamin D receptor null mice. Thus, in the neonatal period, when the skin is phenotypically normal, colony formation by the vitamin D receptor ablated keratinocyte stem cells is normal. However, by 4 weeks of age, a time at which the keratinocyte stem cells of the vitamin D receptor null mice are unable to regenerate a hair follicle *in vivo*, there is dramatic impairment of colony formation *in vitro*. Both of these abnormalities are prevented by expression of a keratinocyte-specific vitamin D receptor transgene in the vitamin D receptor null background [17].

The keratinocyte stem cells that reside in the bulge of the hair follicle express the cell surface markers CD34 and α-6 integrin [18]. Immunohistochemical studies demonstrated that the bulge of the vitamin D receptor null mice exhibited normal immunoreactivity at 4 weeks of age, however by 9 months of age, a distinct bulge could no longer be appreciated. This raised the question as to whether there was a decrease in the number of keratinocyte stem cells with age in the vitamin D receptor null mice. Flow cytometric analyses were performed to quantiate the number of double positive cells. At 1 month of age, the number of cells was not significantly different in the vitamin D receptor null mice compared to that of their wildtype littermates. This suggested that there was a defect in lineage determination of these cells, based on their inability to regenerate a hair follicle *in vivo* and to form large colonies *in vitro*, despite their apparently normal number. However, by 3.5 months of age, there was a significant decrease in the number of double labeled cells in the knockout mice, which was further decreased at 9 months of age. Consistent with normalization of the phenotype of the vitamin D receptor null mice with keratinocyte-specific vitamin D receptor expression, the number of doubly labeled cells in these mice did not differ from those of wildtype controls [17]. Thus these studies suggest both a lineage progression defect in the keratinocyte stem cells of the vitamin D receptor ablated mice, which is evident by 4 weeks of age, as well as a self-renewal defect leading to a decrease in the number of these cells with age.

The canonical Wnt signaling pathway has been shown to play a critical role in the development of the hair follicle as well as in the regulation of postnatal hair cycles [19,20]. Overexpression of a dominant negative Lef1 transgene in keratinocytes leads to alopecia accompanied by the presence of large dermal cysts, a phenotype reminiscent of that observed in the vitamin D receptor null mice [21,22]. Investigations were undertaken to evaluate whether absence of a functional vitamin D receptor altered canonical Wnt signaling. While co-transfection of a vitamin D receptor expression vector, along with β-catenin and Lef1, into COS-7 cells, did not alter the expression of a Wnt reporter, the cooperative interactions of β-catenin and Lef1, which were observed in reporter gene assays in wildtype keratinocytes, were absent in keratinocytes lacking the vitamin D receptor [17]. Thus these investigations suggest that the vitamin D receptor is critical for optimal Wnt signaling in keratinocytes and that absence of this effect may contribute to the keratinocyte stem cell defect observed.

The defined role of the canonical Wnt signaling pathway in the development of the hair follicle and the lack of requirement for the vitamin D receptor during this phase, raise interesting questions as to what additional co-factors or pathways interact with the vitamin D receptor. Mice with keratinocyte-specific ablation of RXR-α, the major RXR isoform in the skin, develop alopecia [23,24], as do mice lacking the nuclear receptor co-repressor hairless [25], the latter of which has been shown to interact with the canonical Wnt signaling pathway [26]. Thus, elucidation of the molecular partners and downstream targets of the unliganded vitamin D receptor in the bulge stem cell is likely to reveal novel roles and mechanisms of action of this nuclear receptor.

Acknowledgment

This work was supported by a grant from the National Institutes of Health DK46974.

References

[1] D.D. Bikle, S. Pillai, Vitamin D, calcium, and epidermal differentiation, Endocr. Rev. 14 (1) (1993) 3–19.
[2] R.G. Erben, D.W. Soegiarto, K. Weber, U. Zeitz, M. Lieberherr, R. Gniadecki, G. Moller, J. Adamski, R. Balling, Deletion of deoxyribonucleic acid binding domain of the vitamin D receptor abrogates genomic and nongenomic functions of vitamin D, Mol. Endocrinol. 16 (7) (2002) 1524–1537.
[3] Y.C. Li, A.E. Pirro, M. Amling, G. Delling, R. Baron, R. Bronson, M.B. Demay, Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia, Proc. Natl. Acad. Sci. U.S.A. 94 (18) (1997) 9831–9835.
[4] P.J. Malloy, J.W. Pike, D. Feldman, The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets, Endocr. Rev. 20 (2) (1999) 156–188.
[5] S.J. Van Cromphaut, M. Dewerchin, J.G. Hoenderop, I. Stockmans, E. Van Herck, S. Kato, R.J. Bindels, D. Collen, P. Carmeliet, R. Bouillon, G. Carmeliet, Duo-

denal calcium absorption in vitamin D receptor-knockout mice: functional and molecular aspects, Proc. Natl. Acad. Sci. U.S.A. 98 (23) (2001) 13324–13329.
[6] T. Yoshizawa, Y. Handa, Y. Uematsu, S. Takeda, K. Sekine, Y. Yoshihara, T. Kawakami, K. Alioka, H. Sato, Y. Uchiyama, S. Masushige, A. Fukamizu, T. Matsumoto, S. Kato, Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning, Nat. Genet. 16 (4) (1997) 391–396.
[7] R. Bouillon, G. Carmeliet, L. Verlinden, E. van Etten, A. Verstuyf, H.F. Luderer, L. Lieben, C. Mathieu, M. Demay, Vitamin D and human health: lessons from vitamin D receptor null mice, Endocr. Rev. 29 (6) (2008) 726–776.
[8] Y. Sakai, M.B. Demay, Evaluation of keratinocyte proliferation and differentiation in vitamin D receptor knockout mice, Endocrinology 141 (6) (2000) 2043–2049.
[9] Z. Xie, L. Komuves, Q.C. Yu, H. Elalieh, D.C. Ng, C. Leary, S. Chang, D. Crumrine, T. Yoshizawa, S. Kato, D.D. Bikle, Lack of the vitamin D receptor is associated with reduced epidermal differentiation and hair follicle growth, J. Invest. Dermatol. 118 (1) (2002) 11–16.
[10] A. Dlugosz, The Hedgehog and the hair follicle: a growing relationship, J. Clin. Invest. 104 (7) (1999) 851–853.
[11] C. Blanpain, W.E. Lowry, A. Geoghegan, L. Polak, E. Fuchs, Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche, Cell 118 (5) (2004) 635–648.
[12] Y. Sakai, J. Kishimoto, M. Demay, Metabolic and cellular analysis of alopecia in vitamin D receptor knockout mice, J. Clin. Invest. 107 (2001) 961–966.
[13] C. Chen, Y. Sakai, M. Demay, Targeting expression of the human vitamin D receptor to the keratinocytes of vitamin D receptor null mice prevents alopecia, Endocrinology 142 (2001) 5386–5389.
[14] K. Skorija, M. Cox, J.M. Sisk, D.R. Dowd, P.N. MacDonald, C.C. Thompson, M.B. Demay, Ligand-independent actions of the vitamin D receptor maintain hair follicle homeostasis, Mol. Endocrinol. 19 (4) (2005) 855–862.
[15] R.J. Morris, C.S. Potten, Slowly cycling (label-retaining) epidermal cells behave like clonogenic stem cells in vitro, Cell Prolif. 27 (5) (1994) 279–289.
[16] W.Y. Wu, R.J. Morris, Method for the harvest and assay of in vitro clonogenic keratinocytes stem cells from mice, Methods Mol. Biol. 289 (2005) 79–86.
[17] L. Cianferotti, M. Cox, K. Skorija, M.B. Demay, Vitamin D receptor is essential for normal keratinocyte stem cell function, Proc. Natl. Acad. Sci. U.S.A. 104 (22) (2007) 9428–9433.
[18] C.S. Trempus, R.J. Morris, C.D. Bortner, G. Cotsarelis, R.S. Faircloth, J.M. Reece, R.W. Tennant, Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34, J. Invest. Dermatol. 120 (4) (2003) 501–511.
[19] U. Gat, R. DasGupta, L. Degenstein, E. Fuchs, De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin, Cell 95 (5) (1998) 605–614.
[20] C. Lo Celso, D.M. Prowse, F.M. Watt, Transient activation of beta-catenin signalling in adult mouse epidermis is sufficient to induce new hair follicles but continuous activation is required to maintain hair follicle tumours, Development 131 (8) (2004) 1787–1799.
[21] B.J. Merrill, U. Gat, R. DasGupta, E. Fuchs, Tcf3 and Lef1 regulate lineage differentiation of multipotent stem cells in skin, Genes Dev. 15 (13) (2001) 1688–1705.
[22] C. Niemann, D.M. Owens, J. Hulsken, W. Birchmeier, F.M. Watt, Expression of DeltaNLef1 in mouse epidermis results in differentiation of hair follicles into squamous epidermal cysts and formation of skin tumours, Development 129 (1) (2002) 95–109.
[23] M. Li, H. Chiba, X. Warot, N. Messaddeq, C. Gerard, P. Chambon, D. Metzger, RXR alpha ablation in skin keratinocytes results in alopecia and epidermal alterations, Development 128 (2001) 675–688.
[24] M. Li, A. Indra, X. Warot, J. Brocard, N. Messaddeq, S. Kato, D. Metzger, P. Chambon, Skin abnormalities generated by temporally controlled RXR alpha mutations in mouse epidermis, Nature 407 (2000) 633–636.
[25] S.J. Mann, Hair loss and cyst formation in hairless and rhino mutant mice, Anat. Rec. 170 (4) (1971) 485–499.
[26] G.M. Beaudoin Jr., J.M. Sisk, P.A. Coulombe, C.C. Thompson, Hairless triggers reactivation of hair growth by promoting Wnt signaling, Proc. Natl. Acad. Sci. U.S.A. 102 (41) (2005) 14653–14658.