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ROLE OF THE VITAMIN D RECEPTOR IN HAIR FOLLICLE BIOLOGY

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Abstract

The vitamin D receptor (VDR) is expressed in numerous cells and tissues, including the skin. The critical requirement for cutaneous expression of the VDR has been proven by investigations in mice and humans lacking functional receptors. These studies demonstrate that absence of the VDR leads to the development of alopecia. The hair follicle is formed by reciprocal interactions between an epidermal placode, which gives rise to the hair follicle keratinocytes and the underlying mesoderm which gives rise to the dermal papilla. Hair follicle morphogenesis ends the second week of life in mice. Studies in VDR null mice have failed to demonstrate a cutaneous abnormality during this period of hair follicle morphogenesis. However, VDR null mice are unable to initiate a new hair cycle after the period of morphogenesis is complete, therefore, do not grow new hair. Investigations in transgenic mice have demonstrated that restricted expression of the VDR to keratinocytes is capable of preventing alopecia in the VDR null mice, thus demonstrating that the epidermal component of the hair follicle requires VDR expression to maintain normal hair follicle homeostasis. Studies were then performed to determine which regions of the VDR were required for these actions. Investigations in mice lacking the first zinc finger of the VDR have demonstrated that they express a truncated receptor containing an intact ligand binding and AF2 domain. These mice are a phenocopy of mice lacking the VDR, thus demonstrate the critical requirement of the DNA binding domain for hair follicle

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homeostasis. Transgenic mice expressing VDRs with mutations in either the ligand-binding domain or the AF2 domain were generated. These investigations demonstrated that mutant VDRs incapable of ligand-dependent transactivation were able to prevent alopecia. Investigations are currently underway to define the mechanism by which the unliganded VDR maintains hair follicle homeostasis.

Keywords

Nuclear Receptor; hair follicle; transgene; knockout; anagen

Vitamin D and its receptor have been shown to have a number of effects on cutaneous homeostasis [1]. 1,25-dihydroxyvitamin D has been shown to inhibit keratinocyte proliferation and to stimulate differentiation of these cells in a dose – dependent manner. Vitamin D analogs have also been used clinically to treat psoriasis, a cutaneous disorder associated with abnormalities in keratinocyte proliferation and differentiation. In addition to its effects on epidermal differentiation, the Vitamin D receptor (VDR) has been shown to be essential for hair follicle integrity. VDR knockout mice [2–5] and humans with mutations in the VDR develop alopecia totalis [6].

Neonatal keratinocytes isolated from VDR null mice were studied to determine if they exhibited abnormalities that could explain the hair loss phenotype observed. These investigations demonstrated that, while proliferation of wild-type keratinocytes was inhibited by 10^{-8} M 1,25-dihydroxyvitamin D, proliferation of the keratinocytes isolated from the VDR null mice was not affected by addition of ligand [7]. However, the expression of markers of keratinocyte differentiation, including keratin, involucrin and loricrin were unaffected by the absence of the VDR. Interestingly, investigations by Xie et al, using immunohistochemistry, demonstrated a decrease in expression of involucrin and loricrin between birth and 3 weeks of age, suggesting that there was an age-dependent decrease in epidermal differentiation in the VDR null mice, similar to the age-dependent hair loss [8].

The hair follicle is an organ composed of epidermal keratinocytes and mesodermal dermal papilla cells. Development of the hair follicle begins at embryonic day 14.5 in the mouse and is dependent on reciprocal interactions between the epithelial placode and the underlying dermal condensate that is mesodermal in origin [9]. In mice, the morphogenic period extends until the third week of postnatal life, which marks the end of the first hair cycle. This first hair cycle, therefore, represents completion of embryological development of the hair follicle. The maintenance of normal hair postnatally is dependent on the integrity of the dermis, epidermis and normal hair cycles. Although many genes have been shown to play a role in hair follicle development, the contribution of these factors to normal postnatal hair cycling has not been established. However, the apparently normal hair morphogenesis that is observed with mutations of the VDR [7,10] as well as with mutations in the gene encoding the nuclear co-repressor Hairless [11,12], suggest that neither of these genes is essential for hair follicle morphogenesis. Notably, mutations of these genes, both in humans [13,14] and in mice, leads to the development of alopecia totalis, suggesting that not all genes required for postnatal hair cycling are essential for normal hair follicle development.

During the hair cycle, the hair follicle retains the dermal papilla, sebaceous glands and upper outer root sheath, including the bulge. The lower part of the follicle goes through periods of growth (anagen), regression (catagen) and rest (telogen). The VDR is expressed in the two major cell populations that make up the hair follicle: the mesodermal dermal papilla cells and the epidermal keratinocytes. VDR expression in the hair follicle is increased during late anagen and catagen, correlating with decreased proliferation and increased differentiation of the keratinocytes [15].

Although we have demonstrated that there is no detectable defect in proliferation of keratinocytes isolated from neonatal VDR ablated mice and that their acquisition of markers of keratinocyte differentiation is normal, subsequent investigations revealed that the VDR null keratinocytes were not capable of responding to anagen initiation after the morphogenic period (the third week of life) [7]. This raised the question as to which population of cells that give rise to the hair follicle require expression of the VDR for normal function. While keratinocytes are easily isolated from the skin of neonatal mice, isolation of a pure population of dermal papilla cells is impracticable, since they are ensheathed by surrounding mesoderm. The VDR null mice were, therefore, crossed to a line of mice expressing Green Fluorescent Protein (GFP) under the control of the versican promoter, the cutaneous expression of which is limited to the dermal papilla in postnatal life [16]. This permitted the isolation of a pure population of dermal papilla cells, using fluorescence activated cell sorting, from mice expressing and lacking the VDR. Hair reconstitution assays were then performed in nude mice. Implantation of a mixture of activated keratinocytes and dermal papilla cells in this model, recapitulates hair follicle morphogenesis. Analogous to the apparently normal development of hair follicles in the VDR null mice, VDR expression in neither of these cell populations was required to form hair follicles. However, when response to anagen initiation was examined, follicles reconstituted with VDR null keratinocytes were found to be unresponsive. However, the VDR status of the dermal papilla cells was inconsequential [7]. These studies demonstrated that VDR expression in keratinocytes was required for normal post-morphogenic anagen initiation. Since these studies were performed in a nude mouse host, they could not address whether potential systemic consequences of VDR ablation contributed to the development of alopecia. To determine whether VDR expression restricted to keratinocytes was sufficient to maintain cutaneous homeostasis, transgenic mice with targeted expression of the VDR to keratinocytes were engineered and bred into the VDR null background. Investigations in VDR ablated mice, expressing the VDR in epidermal keratinocytes demonstrated no impairment in postnatal hair cycles [17]. These investigations definitively demonstrated that VDR expression in keratinocytes was both necessary and sufficient for normal postnatal hair cycling. They also raised the question as to what functional domains of the VDR were required for normal post-morphogenic hair cycles.

VDR null mice, generated by targeted ablation of the second zinc finger, express no detectable receptor protein [3]. However, those generated by targeted ablation of the first zinc finger express a truncated receptor lacking a DNA binding domain, but with intact ligand binding and AF2 domains [2]. Both lines of mice develop alopecia, demonstrating that VDR-DNA interactions are critical for the maintenance of post-natal hair cycles. To address whether ligand binding and/or recruitment of nuclear receptor co-modulators by the VDR is required for cutaneous homeostasis, transgenic mice with targeted expression of mutant VDRs to epidermal keratinocytes were engineered. Mice expressing the mutant VDR transgenes were bred into the VDR null background to permit *in vivo* analyses of the effects of these mutant VDRs on cutaneous homeostasis. The mutation chosen to abolish ligand binding resulted in an L²³³S substitution in the human VDR. To impair recruitment of nuclear receptor co-modulators, a mutation that resulted in a L⁴¹⁷S substitution in the human VDR was engineered. Transient gene expression assays in COS-7 cells and in keratinocytes isolated from VDR null mice expressing either of these transgenes confirmed that they were incapable of mediating ligand-dependent transactivation [18].

Characterization of the effects of these mutant VDRs was performed in both VDR ablated mice, as well as in their heterozygous and wildtype control littermates. No evidence of dominant negative effects were seen in the control littermates, demonstrating lack of interference by the mutant VDRs. The phenotype of the VDR knockout mice expressing the L²³³S transgene was indistinguishable from that of wild-type mice, demonstrating that the effects of the VDR on hair follicle homeostasis do not require ligand binding or ligand-

dependent transactivation. The phenotype of the VDR null mice expressing the L⁴¹⁷S transgene was more complex. While initial analyses demonstrated a normal response to anagen initiation three weeks postnatally, progressive hair loss was observed with age. This was accompanied by the appearance of lipid –laden dermal cysts, analogous to those observed in the transgene negative VDR null mice [18]. These studies supported initial observations that suggested that the absence of hormone and the absence of receptor result in different cutaneous phenotypes. They demonstrate that the effects of the VDR that maintain hair follicle homeostasis are ligand-independent and suggest that recruitment of novel nuclear receptor comodulators by the VDR is required for maintenance of hair follicle homeostasis.

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