Interactions of the Vitamin D Receptor with the Corepressor Hairless

ANALYSIS OF HAIRLESS MUTANTS IN ATRICHLA WITH PAPULAR LESIONS

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Atrichia with papular lesions (APL) and hereditary vitamin D-resistant rickets have a similar congenital hair loss disorder caused by mutations in hairless (HR) and vitamin D receptor (VDR) genes, respectively. HR is a VDR corepressor, and it has been hypothesized that VDR-HR suppress gene expression during specific phases of the hair cycle. In this study, we examined the corepressor activity of HR mutants (E583V, C622G, N970S, V1056M, D1012N, V1136D, and Q1176X) previously described as the molecular cause of APL as well as HR variants (P69S, C397Y, A576V, E591G, R620Q, T1022A) due to non-synonymous polymorphisms in the HR gene. We found that the corepressor activities of all but one of the pathogenic HR mutants were completely abolished. HR mutant E583V exhibited normal corepressor activity, suggesting that it may not be pathogenic. In co-immunoprecipitation assays, all of the pathogenic HR mutants bound VDR but exhibited reduced binding to histone deacetylase 1 (HDAC1), suggesting that the impaired corepressor activity is due in part to defective interactions with HDACs. The HR variants exhibited two classes of corepressor activity, those with normal activity (C397Y, E591G, R620Q) and those with partially reduced activity (P69S, A576V, T1022A). All of the variants interacted with VDR and HDAC1 with the exception of P69S, which was degraded. When coexpressed with VDR, all of the HR pathogenic mutants and variants increased the level of VDR protein, demonstrating that this function of HR was not impaired by these mutations. This study of HR mutations provides evidence for the molecular basis of APL.

The hairless gene product HR is a nuclear protein with a molecular mass of 127 kDa that is expressed in many tissues, including skin and brain (1). HR has been shown to function as a corepressor of multiple nuclear receptors, including thyroid hormone receptor (TR), the retinoic acid receptor-related orphan receptors (ROR) α, β, and γ and the vitamin D receptors (VDR). HR interacts with histone deacetylases (HDACs) and was shown to mediate transcriptional repression via unliganded TR (2, 3). HR also repressed the transcriptional activity of constitutively active RORα. In the case of VDR, coexpression of rat HR in COS-7 African green monkey kidney cells repressed VDR-mediated gene transactivation by both unliganded and ligand-bound receptor (4, 5). A direct HR-VDR interaction and HR corepressor activity on calcitriol-mediated transactivation by VDR were also demonstrated in human keratinocytes (5).

Naturally occurring mutations in the HR gene cause the rare autosomal recessive disease atrichia with papular lesions (APL; OMIM 209500) or alopecia universalis congenita (AUC; OMIM 203655) (6–30). APL and AUC patients exhibit complete hair loss shortly after birth that is irreversible (6, 8, 31). APL can be distinguished clinically from AUC by the presence of a diffuse papular rash in the former (31). A number of mutations have been identified in the HR gene as the molecular basis of APL/AUC in patients of different ethnic origins (8–10, 21). HR knock-out mouse models reveal that HR regulates the timing of epithelial cell differentiation in both the epidermis and hair follicle (32).

Hereditary vitamin D-resistant rickets (HVDRR; OMIM 277440) is a rare recessive genetic disease caused by heterogeneous mutations in the VDR gene (33). The VDR is a member of the steroid-thyroid-retinoid receptor superfamily of ligand-activated nuclear transcription factors. The active form of vitamin D, 1,25-dihydroxyvitamin D3 (calcitriol), binds to the VDR, leading to either the activation or suppression of gene transcription. Interestingly, some patients with HVDRR, those with premature stop mutations, mutations in the DNA binding domain, or mutations that affect heterodimerization with retinoid X receptor (RXR), also present with alopecia and sometimes with skin lesions similar to APL. The clinical and histological findings of hair loss in HVDRR patients and APL patients are strikingly similar despite distinct genetic defects (19, 34). Histologic examination shows a loss of hair follicles and formation of dermal cysts filled with cornified material (19, 34). The shared hair loss phenotype in HVDRR and APL patients suggests that both HR and VDR may impact a common signaling pathway in normal hair growth.

The importance of HR and VDR in hair follicle development has been further demonstrated in mouse models. Targeted
expression in keratinocytes of VDR knock-out mice of either the wild type (WT) VDR or the VDR with mutations in the ligand binding domain restores normal hair follicle cycling (35–37). On the other hand, transgenic expression of HR in progenitor keratinocytes rescues hair follicle regeneration in HR null mice (38). A recent report linked VDR function with HR in the hair follicle by showing that disruption of hair follicle structure during the first catagen phase in VDR null mice was associated with increased expression of HR (39). On the other hand, increased expression of VDR was also found in the HR mutant mice (40), seemingly reflecting the coordinated expression of these two genes involved in the same genetic pathway.

In the present study we investigated whether HR mutations reported to cause APL affected HR corepressor activity with VDR. We demonstrated that these HR mutations have impaired VDR corepressor activity that is associated with defective interaction with HDACs.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—African green monkey kidney cells (COS-7) were obtained from American Type Culture Collection (Rockville, MD) and grown in Dulbecco’s modified Eagle’s medium containing 10% bovine growth serum, (HyClone Laboratories, Inc., Logan, UT) and appropriate antibiotics. Cells were incubated at 37 °C under a 5% CO₂ atmosphere.

**Site-directed Mutagenesis and Plasmid Construction**—Site-directed mutagenesis of the WT human HR cDNA in p3xFlagCMV7.1 (kindly provided by Dr. Axel Hillmer) was performed using the Gene Editor (Promega, Madison, WI) or the QuikChange II XL site-directed mutagenesis kits (Stratagene, La Jolla, CA) following the manufacturer’s instructions. The mutant oligonucleotides used are listed in supplemental Table S1. All clones were sequenced to confirm the presence of the mutation. HA-HDAC1 was constructed by subcloning the mouse HDAC1 cDNA (kindly provided by Dr. Stuart Schreiber) into the Smal sites of hemagglutinin (HA)-tagged phCMV3 (Genlantis, San Diego, CA).

**Transactivation**—COS-7 cells were grown to 60–80% confluence in 12-well tissue culture plates. Cells were transfected with either 62.5 ng of WT VDR with or without 250 ng of human WT HR or HR mutant expression plasmids and 250 ng of 24-hydroxylase (CYP24) promoter VDRE-luciferase plasmid using Polyfect (Qiagen, Valencia, CA). pRLnull Renilla luciferase plasmid (5 ng; Promega) served as an internal control for transfection efficiency. After a 24-h transfection, the cells were incubated overnight in Dulbecco’s modified Eagle’s medium containing 10% bovine growth serum (HyClone Laboratories, Inc., Logan, UT) and appropriate antibiotics. Cells were incubated at 37 °C under a 5% CO₂ atmosphere.

**RESULTS**

In this study we examined the corepressor activity of the WT, APL/AUC mutant, and polymorphic variants of the human HR on calcitriol-mediated transactivation of the CYP24A1 promoter, a known target of VDR. HR corepressor activity was examined in COS-7 cells that express low levels of endogenous VDR. Cells were co-transfected with VDR and HR cDNA expression vectors and a CYP24A1 promoter-luciferase reporter construct and then treated with graded concentrations of calcitriol. In Fig. 1, we show the calcitriol-induced VDR transactivation measured with or without co-transfected WT HR. As expected, calcitriol greatly enhanced transcriptional activity mediated by VDR in the absence of HR. Co-transfection with HR, on the other hand, resulted in a marked reduction in transcriptional activation of the CYP24A1 promoter by the liganded VDR (Fig. 1). However, the repressive effect of HR on VDR transactivation activity was gradually relieved with increasing calcitriol concentrations. Additionally, in the absence of calcitriol, HR suppressed basal VDR transcriptional activity (Fig. 1B).

Our results are consistent with previous reports that HR suppressed both basal and liganded VDR transcriptional activity (4, 5). The reduced repressive activity of HR at high concentrations of calcitriol may be caused by the dissociation of HR from VDR by high concentrations of calcitriol or by the recruitment of co-activators to the liganded VDR (5).

A number of different mutations in HR have been described in patients with APL/AUC. Mutations include both premature stop mutations as well as missense mutations. To assess the impact of these HR mutations on HR corepressor activity, seven mutations in HR that cause APL/AUC (8, 12, 15–17, 19, 22) were recreated by site-directed mutagenesis (Fig. 2, Table 1).
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The corepressor activity of the mutant HRs was then examined in VDR-mediated transactivation assays in COS-7 cells.Calcitriol-induced VDR transactivation of CYP24A1 promoter activity was measured in the absence or presence of co-transfected WT or mutant HRs. Here, the corepressor activity of WT HR on VDR transcrip tional activity as described in Fig. 1 served as a second control in addition to cells transfected with VDR alone.

Of seven HR mutations examined, six had complete loss of their corepressor activity (C622G, N970S, V1056M, D1012N, V1136D, and Q1176X) (Fig. 3A). These data demonstrate that mutations in HR that cause APL/AUC alter its ability to function as a VDR corepressor. The HR mutant E583V was the only so-called pathogenic mutant that exhibited normal corepressor activity. The E583V mutant may be a polymorphism rather than a disease-causing mutation. Alternatively, it is possible that this mutant may exhibit defective corepressor activity on different target genes. Unexpectedly, several of the HR mutants (C622G, N1012D, V1136D, and Q1176X) enhanced VDR transactivation (Fig. 3A) compared with cells transfected with VDR and control vector. At the highest concentration of calcitriol the differences in transactivation are probably not relevant and not convincingly significant.

The effect of pathogenic mutants on VDR basal (no calcitriol) transcri p tional activity was also examined in co-transfected COS-7 cells in the absence of calcitriol. As shown in Fig. 3B, the HR mutants also exhibited defective corepressor activity on VDR basal transactivation activity with the exception of the E583V mutant, which exhibited full repressive activity similar to the WT HR, again indicating it may not be pathogenic.

In addition to the pathogenic mutations described in APL patients, a number of polymorphisms in the HR gene were identified in a systematic mutation screening of HR in androgenic alopecia (8, 44, 45). Several of these polymorphisms result in non-synonymous changes, thereby altering the amino acid sequence (Fig. 2, Table 2). We recreated these polymorphic variants in the HR cDNA and examined their effects on VDR corepressor activity. In contrast to pathogenic mutants described above, the polymorphic variants showed normal or partially reduced corepressor activity on VDR transactivation (Fig. 4A). Three N-terminal polymorphisms, C397Y, E591G, and R620Q, fully retained their repressive ability while three others, P69S, A576V, and T1022A, exhibited partial repressive activity. The T1022A mutation was initially described in a patient with AUC as the molecular cause of the disease (6). Later, another group characterized the mutation as a polymorphic variant (8). In our study, the T1022A mutant exhibited ~50% lower corepressor activity than WT HR (Fig. 4). A second polymorphism, A576V, also showed partially reduced corepressor activity.

The corepressor activity of the polymorphic variants on basal (no calcitriol) VDR transcrip tional activity was also similar to that of liganded VDR (Fig. 4B). P69S, T1022A, and A576V exhibited partially reduced VDR basal transactivation activities as well.

We next examined the expression levels of the HR mutants and variants in COS-7 cells. All of the HR mutants and variants except P69S were expressed at equal levels (Fig. 5). On the
Western blot, the FLAG epitope of the P69S polymorphic variant showed a truncated protein suggesting that this HR variant is unstable and is degraded. HR P69S was also degraded when expressed in HeLa cells (data not shown), demonstrating that its degradation was not unique to COS-7 cells. The proteolysis of this protein most likely contributed to its reduced corepressor activity (Fig. 4).

Next, we examined the corepressor activity of the pathogenic HR mutants and polymorphic variants on transrepression by RORγ. COS-7 cells were co-transfected with RORγ cDNA expression vectors and a RORE-luciferase reporter construct.
struct. A 60% reduction in constitutive ROR transactivation activity was observed when WT HR was coexpressed (Fig. 6A). The pathogenic mutants C622G, N970S, D1012N, V1056M, V1136D, and Q1176X all exhibited defective corepressor activity with RORα. The V1056M mutant exhibited slightly lower expression and modest corepressor activity in these assays (Fig. 6B). The normally expressed HR exon 17 splice variant (HRΔ1072–1126) that we recently described3 that exhibited no corepressor activity with VDR also showed no corepressor activity with RORα. The HR mutant C622G increased RORα transactivation by ~1.5-fold. Of the polymorphic variants, E583V, E591G, and R620Q exhibited normal corepressor activity. P69S that again was degraded (Fig. 6B) increased RORα transactivation ~1.5-fold. HRs C397Y and A576V exhibited reduced activity with RORα as with VDR. Interestingly, the T1022A polymorphic variant that showed ~50% reduction in activity with VDR exhibited no corepressor activity with RORα (Fig. 6A).

Most of the pathogenic mutants with impaired activities were located in the highly conserved C-terminal region of HR (except C622G, which is in the putative C2HC4 zinc finger motif), whereas the majority of the polymorphisms occurred in the less conserved N-terminal portion of HR (Fig. 2). This observation is consistent with the previous report in which the C-terminal portion of HR was defined as the domain responsible for the repressive function of this nuclear protein (4). E583V, previously considered a pathogenic mutation, is located in the region of the other polymorphisms and not in either activity domain.

A direct interaction between HR and VDR has been demonstrated (4, 5). Because the mutant HRs and polymorphic variants exhibited varied co-repressor activities with VDR, we next examined whether these differences might be due to defects in VDR-HR interactions. FLAG-tagged HRs and VDR co-transfected into COS-7 cells were immunoprecipitated with an anti-FLAG monoclonal antibody followed by Western analysis with a VDR monoclonal antibody. As shown in Fig. 7, the VDR protein was immunoprecipitated with the WT HR as well as by all mutant HRs and polymorphic variants. No VDR was co-immunoprecipitated from samples transfected with vector or VDR only. HR proteins were also detected in the immunoprecipitates using anti-FLAG antibody with the exception of P69S, the HR that is degraded (data not shown). The VDR interaction with HR is contained within amino acids 750–864 of HR (4). As all the pathogenic mutants and polymorphic variants are located outside the putative HR-VDR binding region (Fig. 2), it is not surprising to see that all HR mutants and variants exhibited normal binding to VDR. An engineered HR mutant, I800A/I801A, in which two isoleucines were mutated to alanine in one of the two thyroid receptor-interacting domains showed weak binding with VDR in co-immunoprecipitation, indicating that these amino acids are also essential for HR-VDR interaction (Fig. 7). The corepressor activity of the HR I800A/I801A mutant was also abolished (data not shown). Because this mutant exhibited weak binding to VDR, this suggests that multiple sites in HR are involved in the binding interaction with VDR.

Because the HR mutants did not exhibit impaired binding to VDR, we postulated that the decreased corepressor activity of the HR mutants might be associated with defective downstream signaling by the RXR-VDR-HR complex to repress gene transactivation. Because HDACs have been shown to mediate transcriptional suppression by HR-TR (2, 3), we examined whether HDACs were involved in VDR transcriptional suppression by HR. HDACs 1, 2, 3, 5, and 7 and expression vectors were co-transfected with VDR and HR in transrepression assays. HDAC1 and 2 potentiated the corepressor activity of VDR and HR by 30–60%, whereas HDACs 3, 5, and 7 exhibited little or no effect (Fig. 8A). We next determined whether the mutant HRs and polymorphic variants affected HDAC-HR interactions using co-immunoprecipitation assays. Because HDAC 1 and 2 exhibited similar activity we tested HDAC binding using HDAC1. FLAG-tagged HR mutants and HA-tagged HDAC1 cDNAs were co-transfected into COS-7 cells and the HR-HDAC1 complexes immunoprecipitated using an anti-FLAG monoclonal antibody. HA-HDAC1 was then detected on Western blots using HA-specific antibodies. As shown in Fig. 8B, HDAC1 was co-immunoprecipitated by the WT HR. The HR mutants, on the other hand, showed varying degrees of reduced HDAC1 interaction. Stronger bands were obtained from interaction of HDAC1 with WT HR and the polymorphic variants. In contrast, no bands or weaker bands were detected from samples coexpressing HDAC1 and the HR pathogenic mutants except for E583V, which exhibited strong interaction with HDAC1. These results suggest that the reduced suppressive activity of HR mutants is at least in part due to the attenuated binding interaction of HDAC1 with the HR mutants. Equivalent HR protein expression in all samples was confirmed on the Western blots (not shown).

In addition to acting as a corepressor, HR has been shown to stabilize RORα protein by a process that may involve the ubiqui-
uitin-proteasome degradation pathway. Interestingly, we found that the VDR protein levels were dramatically increased when coexpressed with WT HR and all of the HR mutants and polymorphic variants in COS-7 cells compared with cells transfected with VDR and vector control (Fig. 9). Calcitriol itself had no influence on VDR levels in these experiments. Interestingly, HR P69S that we showed to be degraded in our system (Fig. 5) also increased VDR protein. Because P69S retained to a certain extent some corepressor activity (Fig. 4), it suggests that the protein degradation occurred after interacting with the VDR. The mechanism underlying the enhancement of VDR protein by HR is currently being investigated.

**DISCUSSION**

Recent studies have shown that HR acts as a corepressor of VDR, working likely through the VDR-RXR-HR complex to control gene expression during the hair cycle (4, 5). However, the precise molecular mechanism underlying the effect of HR on VDR-mediated transcription in the development of APL/AUC is largely unknown. In the present work, the impact of HR mutations causing APL/AUC on VDR-mediated transcription was examined. Six pathogenic mutations (C622G, N970S, D1012N, V1056M, V1136D, and Q1176X) caused complete loss of corepressor activity in both basal and calcitriol-induced transactivation by VDR. One mutation, E583V, identified in a patient with APL (22) exhibited normal corepressor activity, suggesting that this mutation is not a disease-causing mutation.

On the other hand, of six polymorphic variants of HR studied, three (C397Y, E591G, and R620Q) showed normal corepressor activity and three (P69S, A576V, and T1022A) showed partially reduced corepressor activity. The P69S polymorphic variant was degraded, which most likely accounted for its reduced corepressor activity. The A576V polymorphic variant that is located close to an LXXLL (where L is leucine and X is any amino acid) nuclear receptor-interacting motif also exhibited reduced corepressor activity. Although we did not detect any difference in binding to VDR versus the WT HR, this polymorphism may affect interactions with nuclear receptors. The T1022A mutation was originally described as the cause of AUC (6). A later study showed that the T1022A mutation was present in the heterozygous state in 14 individuals in a German population, resulting in an allele frequency of 1.2% that suggested that this was a polymorphism and not a disease-causing mutation.
change may not be disruptive. However, our results show that the T1022A polymorphism exhibits reduced corepressor activity with VDR. It will be interesting to see whether the analogous mutation (A1022T) in the rat or mouse HR causes a similar reduction in corepressor activity. These polymorphisms with reduced activity may increase the risk of APL/AUC or other hair disorders, depending on the activity of other critical genes.

Because HR also functions as a corepressor of RORα, we also examined corepressor activity of the pathogenic HR mutants and polymorphic variants on the constitutive transcriptional activity of RORα (3, 46). Again, in this study we found that all of the HR pathogenic mutants except E583V had absent or severely reduced corepressor activity. Of the polymorphic variants, C376Y and A576V showed partially reduced corepressor activity. The T1022A variant, however, exhibited no corepressor activity, again suggesting it may be a pathogenic mutation. Whether the T1022A polymorphism contributes to some form of hair loss or disease in homozygous individuals is unknown at this time. A larger population study of the HR polymorphisms that exhibit reduced activity may provide answers to this question. Thompson et al. (47) recently showed that several mutations (C642G, N988S, D1030N, and V1154D) in the rat HR corresponding to missense mutations (C622G, N970S, D1012N, and V1136D, respectively) in patients with APL also reduced or abolished corepressor activity with TR.

HR has a two-domain structure. In the central region of HR there is a zinc finger motif that exhibits homology to the C2HC4 DNA binding motif of the GATA family of transcription factors and the rat testis-specific gene A (TSGA). (48). At the C terminus of HR there is a highly conserved region related to JmjC domains (49, 50). Five mutations with impaired corepressor activity were located in the JmjC domain (N970S, D1012N, V1136D, and Q1176X); the other mutation (C622G) occurred in a conserved cysteine in the putative C2HC4 zinc finger. On the other hand, several of the polymorphisms (P69S, C397Y, and A576V) were located in the N-terminal region of HR. Deletion analysis of the HR showed that the C-terminal region (amino acids 568–1207) including the JmjC domain was responsible for the repressive activity of HR (4).
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Taken together, these observations suggest that the JmjC domain contains the corepressor activity of HR. Recently, proteins with JmjC domains have been demonstrated to have histone demethylase activity, and it is thought that the JmjC domain confers this activity (51). The human homolog of TSGA, JHDM2A, also has a JmjC domain and has recently been shown to demethylate the repressive histone mark, histone 3 lysine 9, thereby antagonizing gene silencing (52). The structural domains of HR and JHDM2A are very similar. HR and JHDM2A share the same C2HC4 zinc finger motif and JmjC domains as well as nuclear receptor-interacting domains (48). In fact, the amino acids that are mutated in HR that cause APL are conserved in JHDM2A. Whether the JmjC domain in HR has similar histone demethylase activity is currently under investigation.

HDACs are thought to be a component of the repressor protein complex, acting as an important factor in chromatin remodeling (53–56). Members of the classical HDAC family are divided into two different phylogenetic classes, namely class I (HDAC 1, 2, 3, and 8) and class II (HDAC 4, 5, 6, 7, 9, and 10) on the basis of structural homology (57). HDAC 1, 3, and 5 were investigated. It is likely that multiple HDACs are involved in this further potentiation by these HDACs is currently being investigated. HDAC2 also potentiated HR corepressor activity whereas the corepressor activity of WT HR on VDR-mediated transactivation point. The decreased repressive ability of the HR mutants is at least in part which exhibited normal binding. These results suggest that the decreased repressive activity of the HDAC family compared with that of WT HR with the exception of ES83V, which exhibited normal binding. These results suggest that the decreased repressive activity of the HR mutants is at least in part due to their defective interaction with HDACs. The inhibitory property of HDAC1 was further confirmed by potentiating the corepressor activity of WT HR on VDR-mediated transactivation. HDAC2 also potentiated HR corepressor activity whereas HDACs 3, 5, and 7 exhibited little or no additional effect (Fig. 8). Whether this was due to a lack of an effect on HR or due to an overabundance of these HDACs in COS-7 cells that prevents further potentiation by these HDACs is currently being investigated. It is likely that multiple HDACs are involved in this process. Additional experiments are underway to clarify this point.

We postulate that the total loss of corepressor activity of the HR mutants in APL may lead to the inappropriate expression of a gene or a set of genes whose protein product then disrupts the normal hair cycle process. In fact, HR has been shown to reactivate hair growth in mice by suppressing the expression of Wnt inhibitory signaling molecules such as WISE and Soggy (38, 47). Likewise, VDR mutations that cause HVDRR with alopecia may also relieve gene repression leading to the disruption of the hair cycle.

In summary, we found that the HR mutations causing alopecia showed impaired corepressor activity on VDR-mediated transactivation that is due in part to the attenuated interaction of HR with HDACs. Our results provide evidence for the pathobiological effects of HR mutations in APL/AUC.

REFERENCES

1. Cachon-Gonzalez, M. B., Fenner, S., Coffin, J. M., Moran, C., Best, S., and Stoye, J. P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7717–7721
2. Potter, G. B., Beaudoin, G. M., III, DeRienzo, C. L., Zarach, J. M., Chen, S. H., and Thompson, C. C. (2001) Genes Dev. 15, 2687–2701
3. Potter, G. B., Zarach, J. M., Sisk, J. M., and Thompson, C. C. (2002) Mol. Endocrinol. 16, 2547–2560
4. Hsieh, J. C., Sisk, J. M., Jurutka, P. W., Haussler, C. A., Slater, S. A., Haussler, M. R., and Thompson, C. C. (2003) J. Biol. Chem. 278, 38665–38674
5. Xie, Z., Chang, S., Oda, Y., and Bilek, D. D. (2006) Endocrinology 147, 314–323
6. Ahmad, W., Faiyaz ul Haque, M., Brancolini, V., Tsou, H. C., ul Haque, S., Lam, H., Aita, V. M., Owen, J., deBlaquier, M., Frank, J., Cserhalmi-Friedman, P. B., Leask, A., McGrath, J. A., Peacocke, M., Ahmad, M., Ott, J., and Christiano, A. M. (1998) Science 279, 720–724
7. Ahmad, W., Irvine, A. D., Lam, H., Buckley, C., Bingham, E. A., Panteleyev, A. A., Ahmad, M., McGrath, J. A., and Christiano, A. M. (1998) Am. J. Hum. Genet. 63, 984–991
8. Cichon, S., Anker, M., Vogt, I. R., Rohleder, H., Putzstuck, M., Hillmer, A., Farooq, S. A., Al-Dhafri, K. S., Ahmad, M., Haque, S., Rietelsiel, M., Propping, P., Kruse, R., and Nothen, M. M. (1998) Hum. Mol. Genet. 7, 1671–1679
9. Zlotogorski, A., Ahmad, W., and Christiano, A. M. (1999) Hum. Genet. 103, 400–404
10. Ahmad, W., Nomura, K., McGrath, J. A., Hashimoto, I., and Christiano, A. M. (1999) J. Invest. Dermatol. 113, 281–283
11. Ahmad, W., Zlotogorski, A., Panteleyev, A. A., Lam, H., Ahmad, M., ul Haque, M. F., Abdallah, H. M., Dragan, L., and Christiano, A. M. (1999) Genomics 56, 141–148
12. Kruse, R., Cichon, S., Anker, M., Hillmer, A., Barroso-Nunez, P., Cantu, J. M., Leal, E., Weinlich, G., Schmutz, M., Fritsch, P., Ruzicka, T., Proppping, P., and Nothen, M. M. (1999) J. Invest. Dermatol. 113, 954–959
13. Sprecher, E., Bergman, R., Szargel, R., Friedman-Birnbaum, R., and Cohen, N. (1999) Am. J. Hum. Genet. 64, 1323–1329
14. Sprecher, E., Lestringant, G. G., Szargel, R., Bergman, R., Labay, V., Frossard, P. M., Friedman-Birnbaum, R., and Cohen, N. (1999) J. Invest. Dermatol. 113, 687–690
15. Aita, V. M., Ahmad, W., Panteleyev, A. A., Kozlowska, U., Kozlowska, A., Gilliam, T. C., Jablonska, S., and Christiano, A. M. (2000) Exp. Dermatol. 9, 157–162
16. Henn, W., Zlotogorski, A., Lam, H., Martinez-Mir, A., Zaun, H., and Christiano, A. M. (2002) J. Am. Acad. Dermatol. 47, 519–523
17. Klein, I., Bergman, R., Indelman, M., and Sprecher, E. (2002) J. Invest. Dermatol. 119, 920–922
18. Zlotogorski, A., Martinez-Mir, A., Green, J., Lamdagger, H., Panteleyevagger, A. A., Sinclair, R., and Christiano, A. M. (2002) J. Invest. Dermatol. 118, 881–886
19. Zlotogorski, A., Panteleyev, A. A., Aita, V. M., and Christiano, A. M. (2002) J. Invest. Dermatol. 118, 887–890
20. Indelman, M., Bergman, R., Lestringant, G. G., Peer, G., and Sprecher, E. (2003) Br. J. Dermatol. 148, 553–557
21. Paller, A. S., Varigos, G., Metzker, A., Bauer, R. C., Opie, J., Martinez-Mir, A., Christiano, A. M., and Zlotogorski, A. (2003) J. Invest. Dermatol. 121, 430–432
22. Paradisi, M., Chuang, G. S., Angelo, C., Pedicelli, C., Martinez-Mir, A., and Christiano, A. M. (2003) Clin. Exp. Dermatol. 28, 535–538
23. Djebali, K., Zlotogorski, A., Metzker, A., Ben-Amitai, D., and Christiano, A. M. (2004) Exp. Dermatol. 13, 251–256
24. Ashoor, G. G., Greenstein, R. M., Lam, H., Martinez-Mir, A., Zlotogorski, A., and Christiano, A. M. (2005) J. Invest. Dermatol. Sci. 40, 29–33
25. John, P., Aslam, M., Rafiq, M. A., Amin-ud-din, M., Haque, S., and Ahmad, W. (2005) Arch. Dermatol. Res. 297, 226–230
26. Masse, M., Martinez-Mir, A., Lam, H., Geraghty, M. T., and Christiano, A. M. (2005) Clin. Exp. Dermatol. 30, 363–365
27. Paradisi, M., Masse, M., Martinez-Mir, A., Lam, H., Pedicelli, C., and Christiano, A. M. (2005) Eur. J. Dermatol. 15, 332–338
28. Wali, A., Ansar, M., Khan, M. N., and Ahmad, W. (2006) Clin. Exp. Dermatol. 31, 695–698
29. Betz, R. C., Indelman, M., Pforr, J., Schreiner, F., Bauer, R., Bergman, R., Lentze, M. J., Nothen, M. M., Cichon, S., and Sprecher, E. (2007) Arch. Dermatol. Res. 299, 157–161
30. O'Regan, G. M., Zurada, J., Martinez-Mir, A., Christiano, A. M., and Irvine, A. D. (2007) *Br. J. Dermatol.* **156**, 744–747
31. Sprecher, E., Bergman, R., Szargel, R., Raz, T., Labay, V., Ramon, M., Baruch-Gershoni, R., Friedman-Birnbaum, R., and Cohen, N. (1998) *Am. J. Med. Genet.* **80**, 546–550
32. Zarach, J. M., Beaudoin, G. M., III, Coulombe, P. A., and Thompson, C. C. (2004) *Development* **131**, 4189–4200
33. Zarach, J. M., Beaudoin, G. M., III, Coulombe, P. A., and Thompson, C. C. (2004) *Development* **131**, 4189–4200
34. Miller, J., Djabali, K., Chen, T., Liu, Y., Ioffreda, M., Lyle, S., Christiano, A. M., Holick, M., and Cotsarelis, G. (2001) *J. Investig. Dermatol.* **117**, 612–617
35. Chen, C. H., Sakai, Y., and Demay, M. B. (2001) *Endocrinology* **142**, 5386–5389
36. Kong, J., Li, X. J., Gavin, D., Jiang, Y., and Li, Y. C. (2002) *J. Investig. Dermatol.* **118**, 631–638
37. Skorija, K., Cox, M., Sisk, J. M., Dowd, D. R., MacDonald, P. N., Thompson, C. C., and Demay, M. B. (2005) *Mol. Endocrinol.* **19**, 855–862
38. Beaudoin, G. M., III, Sisk, J. M., Coulombe, P. A., and Thompson, C. C. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 14653–14658
39. Bikle, D. D., Elalieh, H., Chang, S., Xie, Z., and Sundberg, J. P. (2006) *J. Cell. Physiol.* **207**, 340–353
40. Nam, Y., Kim, J. K., Cha, D. S., Cho, J. W., Cho, K. H., Yoon, S., Yoon, J. B., Oh, Y. S., Suh, J. G., Han, S. S., Song, C. W., and Yoon, S. K. (2006) *Genomics* **87**, 520–526
41. Malloy, P. J., Xu, R., Cattani, A., Reyes, L., and Feldman, D. (2004) *J. Bone Miner. Res.* **19**, 1018–1024
42. Malloy, P. J., Zhu, W., Zhao, X. Y., Pehling, G. B., and Feldman, D. (2001) *Mol. Genet. Metab.* **73**, 138–148
43. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
44. Hillmer, A. M., Kruse, R., Betz, R. C., Schumacher, J., Heyn, U., Propping, P., Nothen, M. M., and Cichon, S. (2001) *Am. J. Hum. Genet.* **69**, 235–237
45. Hillmer, A. M., Kruse, R., Macciardi, F., Heyn, U., Betz, R. C., Ruzicka, T., Propping, P., Nothen, M. M., and Cichon, S. (2002) *Br. J. Dermatol.* **146**, 601–608
46. Moraitis, A. N., Giguere, V., and Thompson, C. C. (2002) *Mol. Cell. Biol.* **22**, 6831–6841
47. Thompson, C. C., Sisk, J. M., and Beaudoin, G. M., III (2006) *Cell Cycle* **5**, 1913–1917
48. Knebel, J., De Haro, L., and Janknecht, R. (2006) *J. Cell. Biochem.* **99**, 319–329
49. Clissold, P. M., and Ponting, C. P. (2001) *Trends Biochem. Sci.* **26**, 7–9
50. Trewick, S. C., McLaughlin, P. J., and Allshire, R. C. (2005) *EMBO Rep.* **6**, 315–320
51. Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M. E., Borchers, C. H., Tempst, P., and Zhang, Y. (2006) *Nature* **439**, 811–816
52. Yamane, K., Toumazou, C., Tsukada, Y. I., Erdjument-Bromage, H., Tempst, P., Wong, J., and Zhang, Y. (2006) *Cell* **125**, 483–495
53. Nagy, L., Kao, H. Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L., and Evans, R. M. (1997) *Cell* **89**, 373–380
54. Alland, L., David, G., Shen-Li, H., Potes, J., Muhle, R., Lee, H. C., Hou, H., Jr., Chen, K., and DePinho, R. A. (2002) *Mol. Cell. Biol.* **22**, 2743–2750
55. Jones, P. L., and Shi, Y. B. (2003) *Curr. Top. Microbiol. Immunol.* **274**, 237–268
56. Privalsky, M. L. (2004) *Annu. Rev. Physiol.* **66**, 315–360
57. de Ruijter, A. J., van Gennip, A. H., Caron, H. N., Kemp, S., and van Kuijlenburg, A. B. (2003) *Biochem. J.* **370**, 737–749
58. Ishizuka, T., and Lazar, M. A. (2003) *Mol. Cell. Biol.* **23**, 5122–5131