A Homozygous Nonsense Mutation in the Zinc-Finger Domain of the Human Hairless Gene Underlies Congenital Atrichia

To the Editor:

Congenital atrichia (OMIM 209500) is a rare form of total alopecia and is inherited in an autosomal recessive pattern (Burke, 1954; Landes and Langer, 1956; Ahmad et al, 1993, 1998a, b, 1999; Zlotogorski et al, 1998). In individuals affected with this form of hair loss, hairs are typically absent from the scalp, and patients are almost completely devoid of eyebrows, eyelashes, axillary, and pubic hair. A scalp skin biopsy from affected individuals typically reveals the absence of mature hair follicle structures. Variations in the structure and shape of hair follicle remnants have been reported in patients with congenital atrichia. These include shortened hair follicles containing horny plugs, and a reduced number of pilosebaceous units containing malformed hairs without cuticles (Porter, 1973). Affected individuals show no growth or developmental delay, have normal hearing, teeth, and nails, and no abnormalities in sweating. Congenital atrichia affects males and females equally, from all ethnic backgrounds.

We and others (Ahmad et al, 1998a; Nothen et al, 1998) recently reported a linkage in this form of atrichia to chromosome 8p12, and we identified the human homolog of the hairless gene in the same region of chromosome 8 (Ahmad et al, 1998a). More recently, we have identified pathogenic nonsense and deletion mutations in the human hairless gene in this form of atrichia in families from Pakistan, Ireland, and Israel (Ahmad et al, 1998a, b, 1999; Zlotogorski et al, 1998). As an extension of these studies, here we studied a family from Japan with a single member affected with congenital atrichia, whose clinical history was described in detail earlier by Nomura and Hashimoto (1998). Briefly, the proband was born with normal hair, which began to shed at the age of 6 mo and progressed to complete hairlessness at the age of 1 y. Hair was absent from the scalp, axillae, pubis, and other parts of the body, and eyebrows and eyelashes were sparse. She had the additional characteristics features of grouped cystic and papular lesions on the neck, buttocks, and thighs. A skin biopsy from the neck showed deep dermal keratinous cysts. The patient exhibited no other physical, developmental, or neuropsychologic abnormality. The family pedigree is strongly suggestive of autosomal recessive inheritance (Fig 1a).

To search for an underlying mutation in the hairless gene, genomic DNA was isolated from peripheral blood collected in EDTA-containing tubes according to standard techniques (Sambrook, 1989). Exons and splice junctions were polymerase chain reaction amplified from genomic DNA and sequenced directly in an ABI Prism 310 Automated Sequencer, using the ABI Prism Rhodamine Terminator Cycle Sequencing Ready Reaction Sequencing Kit (PE Applied Biosystems, Foster City, CA), following purification in a Centriflex Gel Filtration Cartridge (Edge Biosystems, Gaithersburg, MD). A 287 bp polymerase chain reaction fragment containing exon 6 of the human hairless gene was amplified using primers described earlier (Ahmad et al, 1998b). Sequence analysis of exon 6 revealed a C-to-T transition at nucleotide position 1837 (Fig 1b), resulting in the conversion of an arginine to a premature termination codon, designated R613X. This mutation is predicted to result in the absence of mRNA and functional protein, due to nonsense-mediated mRNA decay (Maquat, 1996).

Recently, we have reported nonsense and deletion mutations in the human hairless gene in families from Pakistan, Ireland, and Israel with congenital atrichia (Ahmad et al, 1998a, b; Zlotogorski et al, 1998; Ahmad et al, 1999). In this study, we report the identification of a nonsense mutation in the highly conserved zinc-finger DNA binding domain of the gene in a family from Japan. In our previous study, we reported a missense mutation (R620Q) in the zinc-finger domain of the hairless gene in a large family of Irish travellers from Ireland (Ahmad et al, 1998b), suggesting that mutations tend to cluster in the zinc-finger domain of the gene, as shown in our analysis of hairless and rhino mouse mutations (Ahmad et al, 1998c, d; Panteleyev et al, 1998a). The phenotypic appearance of the proband in this family is reminiscent of our previous cases, as well as those reported earlier (Burke, 1954; Landes and Langer, 1956; Cantu et al, 1980), including atrichia with cystic papules on the elbows and knees. As early as 1954, this rare human disease was named atrichia with papular lesions, and was characterized as normal hair formation at birth followed by hair loss associated with the formation of comedones and follicular cysts (Damste and Prakken, 1954; Landes and Langer, 1956; Lowenthal and Prakken, 1961; Del Castillo et al, 1974; Cantu et al, 1980; Kanzler and Rasmussen, 1986; Rook and Dawber, 1991; Mischia et al, 1992). In 1989, the human disease was first proposed as a homolog of the hairless mouse mutation (Sundberg et al, 1989).

The molecular basis of the hairless mouse phenotype was previously found to be the result of a murine leukemia proviral insertion in intron 6 of the hairless gene, reported to result in aberrant splicing (Cachon-Gonzalez et al, 1994). In addition, we recently reported a series of nonsense and deletion mutations in the hairless gene in the rhino mice, which were known to be allelic to hairless mice (Ahmad et al, 1998c, d; Panteleyev et al, 1998a). The hairless protein is a putative transcription factor with a single zinc-finger domain, which is highly expressed in the brain and the skin. The hairless gene contains a single zinc-finger DNA binding domain with a novel spacing of a conserved six-cysteine motif, and only weak homology to two classes of genes: TSGA, a testes-specific transcription factor, and TRIPs, thyroid hormone-interacting proteins (Ahmad et al, 1999). Despite the high levels of hairless expression in the brain as compared with skin, neither hairless nor rhino mice display any neurologic, behavioral, or developmental defects whatsoever. This finding correlates well with human patients with atrichia, in whom we have also never observed these sequelae. We have, however, observed a striking genotype-phenotype relationship between hairless and rhino mice with respect to the severity of the disease phenotype in the hair and skin. The hairless proviral insertion mutation results in a moderate hairless phenotype; however, the nonsense and deletion mutations in rhino mice result in a much more profound and accelerated phenotype (Ahmad et al, 1998c, d; Panteleyev et al, 1998a). Surprisingly, we have not observed this genotype-phenotype relationship in our human patients with atrichia. We have identified two missense and two deletion mutations thus far, with indistinguishable clinical findings. The Japanese patient in this study represents the first nonsense mutation in the hairless gene in an atrichia patient,
and similar to the earlier reports, the phenotype is identical. Why we observe a marked genotype–phenotype correlation in mice but not humans with atrichia is currently under investigation.

The precise function of hairless protein remains elusive; however, recent studies have established that hairless functions as a transcriptional corepressor in the brain, and is regulated directly by thyroid hormone (Thompson, 1996; Thompson and Bottcher, 1997). In the hair follicle, it appears to function in the cellular transition to the first adult hair cycle, and in its absence hair growth completely ceases, a new hair is never induced, and the result is a complete form of inherited alopecia. The hair matrix cells undergo a premature and massive apoptosis, together with a concomitant decline in Bcl-2 expression, a loss of NCAM positivity, and a disconnection with the overlying epithelial sheath essential for the movement of the dermal papilla (Panteleyev et al., 1998b, c). As a consequence, the hair bulb and dermal papilla remain stranded in the dermis, and indispensable messages between the dermal papilla and stem cells in the bulge are not transmitted, thus no further hair growth occurs. In hairless mice and humans with congenital atrichia, we postulate that the absence of hairless protein initiates a premature and abnormal catagen due to abnormal signaling that normally control catagen-associated hair follicle remodeling (Panteleyev et al., 1998c, d). These findings suggest that the hairless gene product may play a crucial role in maintaining the balance between cell proliferation, differentiation, and apoptosis in the hair follicle as well as in the interfollicular epidermis.

Wasim Ahmad, Kazuo Nomura,*, John A. McGrath,† Isao Hashimoto,‡ Angela M. Christiano§ Departments of Dermatology and §Genetics & Development, Columbia University, New York, U.S.A.

*Department of Dermatology, Aomori Prefectural Central Hospital, Aomori, Japan

†St. John’s Institute of Dermatology, St. Thomas’ Hospital, London, U.K.

‡Department of Dermatology, Hirosaki University School of Medicine, Hirosaki, Japan

REFERENCES


UV Induces p21WAF1/CIP1 Protein in Keratinocytes Without p53

To the Editor:

A central effect of UV in the skin is to arrest the cell cycle in G1 and S phase (Epstein et al., 1970; Petrocelli et al., 1996). p21WAF1/CIP1, a cyclin-dependent kinase inhibitor, is induced in cultured keratinocytes and in human skin following UV irradiation (Liu and Pelling, 1995; Ponten et al., 1995; Petrocelli et al., 1996). Induction of this protein is known to mediate G1 and S phase arrest by inhibiting cdk2 and cdk4 kinases (Petrocelli et al., 1996; Poon et al., 1996). p21WAF1/CIP1 also blocks DNA replication by interacting with proliferating-cell nuclear antigen (PCNA) (Li et al., 1994). In addition, recent evidence suggests that it facilitates repair of UV-induced DNA photoproducts (Sheikh et al., 1997). p21WAF1/CIP1 was first identified as a p53-inducible gene (El-Deiry et al., 1993); the tumor suppressor p53 is also known to play an important role in the UV response (Laurencin et al., 1994). Although p21WAF1/CIP1 mRNA induction by DNA-damaging agents typically requires p53 (El-Deiry et al., 1994; Macleod et al., 1995), p21WAF1/CIP1 can also be regulated during normal tissue development and cellular differentiation without p53 (Macleod et al., 1995). In this study, we investigated whether UV induction of p21WAF1/CIP1 protein in keratinocytes requires p53 in vivo and in vitro.

We first took advantage of the recent availability of a p21WAF1/CIP1 antibody effective in paraffin sections of mouse skin, and studied p21WAF1/CIP1 protein expression in vivo. Figure 1 shows that there was detectable p21WAF1/CIP1 expression in unirradiated keratinocytes of wild-type p53 mice, but minimal basal expression in p53-knockout mice. This is consistent with previous evidence that p53 may be required to control the expression of p21WAF1/CIP1 during keratinocyte differentiation (Weinberg et al., 1994; Misero et al., 1996). At 24 h post-UV-irradiation, p21WAF1/CIP1 protein levels were elevated in epidermal keratinocytes of the wild-type p53 mouse, as expected. Strikingly, however, p21WAF1/CIP1 induction by UV occurred to an apparently equal extent in the keratinocytes of p53-deficient mice.

The observation of p21WAF1/CIP1 induction by UV without p53 was unexpected, since other DNA-damaging agents elevate p21WAF1/CIP1 in a p53-dependent manner in vitro (El-Deiry et al., 1994; Macleod et al., 1995). To exclude an in vivo/in vitro difference, we next investigated whether, in vitro, UV induces p21WAF1/CIP1 protein without p53. Figure 2 shows that UV induced the p21 protein level in murine p53-null NHK4 keratinocytes (Azzoli et al., 1998) at 40 J per m2 and 120 J per m2. In addition, p21WAF1/CIP1 mRNA expression was upregulated by UV at 120 J per m2, though not at 40 J per m2. Murine p53-deficient (10)1 fibroblasts (Harney and Levine, 1991) behaved similarly (data not shown).

We here provide evidence that UV, the DNA-damaging agent most relevant to skin, can induce p53-independent p21WAF1/CIP1 protein expression in keratinocytes in vivo and in vitro. This observation may have implications for skin’s protection against basal and squamous cell carcinoma. When keratinocytes are damaged by UV, the p53 tumor suppressor is known to offer protection by inducing cell cycle arrest (“guardian-of-the-genome”) and apoptosis (“guardian-of-the-tissue”) (Lane, 1992; Hall et al., 1993; Liu et al., 1994; Ziegler et al., 1994); however, the p53 gene is itself a target of UV radiation. UV-induced p53 mutations have been found in most human skin cancers, in skin precancers (actinic keratoses), and in 20% of keratinocytes in sun-exposed skin (Jonason et al., 1996; Ziegler et al., 1994). In contrast, there are few p21WAF1/CIP1 mutations in human skin cancers (our unpublished results) or other malignancies (Shiohara et al., 1994).

Therefore, p21WAF1/CIP1 induction by UV in the absence of p53 may compensate for the loss of p53 functions by nevertheless initiating cell cycle arrest, inhibiting DNA replication, and facilitating DNA repair.

Manuscript received April 20, 1998; revised April 1, 1999; accepted for publication April 22, 1999.

Reprint requests to: Dr. Douglas E. Brash, Department of Therapeutic Radiology, HRT 369, Yale University School of Medicine, New Haven, CT 06520-8040. E-mail: douglas.brash@yale.edu

This work was supported by NIH grant CA55737 (D.E.B.) and the Leslie H. Warner Postdoctoral Fellowship in Cancer Research (M.L.). We are very