Cytokine expression was also analyzed in skin-draining lymph nodes (LN; Figure 2l). Upon MC903 treatment, the induction of Th2 cytokines (IL4, IL13, IL10, IL6, and IL31) in WT LNs was not observed in $TSLP^{ep-/-}$ LNs, indicating that the MC903-triggered Th2 response is abolished in $TSLP^{ep-/-}$ mice. No change was seen for IFNγ transcript levels in WT or $TSLP^{ep-/-}$ LNs, from either MC903- or ethanol-treated mice, suggesting that MC903 application does not involve a Th1 response in LNs.

In conclusion, we demonstrate unequivocally here that TSLP produced by keratinocytes is absolutely required in pathogenesis of AD triggered by topical application of the vitamin D3 analogue MC903. We also show that in this AD model, induction of IL4, IL13, IL31, and eotaxin-2 is fully TSLP dependent, whereas that of IL6, IL10, and monocyte chemotactic protein-2 is only partially TSLP dependent, and that of IFNγ is TSLP independent. Finally, our floxed TSLP mice will be helpful for selective ablation of TSLP in other cell types (Soumelis and Liu, 2004; Ziegler and Liu, 2006; Holgate, 2007; Sokol *et al.*, 2008), and therefore to further elucidate the physiological and pathological function of this cytokine.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Methods
Figure S1. Comparison of cytokine and chemokine expression in ears of ethanol or MC903-treated wild-type (WT) and $TSLP^{-/-}$ null (germline mutation of TSLP) mice.

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Identification of XP Complementation Groups by Recombinant Adenovirus Carrying DNA Repair Genes

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TO THE EDITOR

Individuals with xeroderma pigmentosum syndrome (XP) present an extremely high sensitivity to sunlight, with increased incidence of skin cancer (especially in sun-exposed areas), when compared to the normal population. Some XP patients can also display developmental complications and early aging features.

A genetic defect in the nucleotide excision repair pathway (NER) was described as the molecular cause of XP syndrome (Cleaver, 1968), and somatic cell fusion accompanied by

Abbreviations: FHN, normal human fibroblast; NER, nucleotide excision repair; UDS, unscheduled DNA synthesis; UV, ultraviolet; XP, xeroderma pigmentosum

functional complementation using unscheduled DNA synthesis (UDS) assays led to the identification of seven complementation groups, which correspond to some of the genes involved in NER (designated *XPA* to *XPG*) (Costa *et al.*, 2003; Lehmann, 2003). Among these genes, mutations in *XPA* and *XPC* are the most frequent and are responsible for approximately half of all XP cases in the world (Zeng *et al.*, 1997). All described XP mutations can be found in the XP mutations database (http://xpmutations.org).

This work describes mutations in fibroblasts obtained from three children with clinical diagnosis of XP, from two nonrelated families. All three patients were born from consanguineous marriages and manifested sunburning on minimal sun exposure, photophobia, and corneal and eye lid deformities. None of them presented neurological abnormalities. The XP02SP patient is 12 years old and displayed several cutaneous tumors in sun-exposed areas, beginning at age 7 (basal-cell carcinomas, squamous-cell carcinoma, malignant fibrohistiocytoma, and atypical *in situ* melanocyte proliferation). The XP03SP patient is also a 12-year old, and developed actinic keratosis and basal-cell carcinomas at the age of 8 years. Patient XP04SP, brother of patient XP03SP, died at age 13 after complications of an invasive and extremely agressive squamous-cell carcinoma that led to partial destruction of his face. This patient also had a T-cell lymphoma at the age of 3, curiously not commonly observed in XP patients, which responded to chemotherapy, although an acceleration of the progression of skin lesions was observed during treatment.

To determine XP patients' complementation group, fibroblasts from skin biopsies of patients were infected with recombinant adenovirus carrying *XPA* (AdyXPA) or *XPC* (AdXPC; Muotri *et al.*, 2002) functional cDNA and, later, gene complementation was analyzed by UDS. For noninfected cells, UV-irradiated NER-proficient fibroblasts presented a high number of nuclear grains, whereas cells from patients exhibited reduced UDS levels, confirming their XP diagnoses. Cells infected with AdyXPA (Figure 1a) did not present any increase in grain number. In contrast, after the XP fibroblasts were infected with AdXPC, the levels of UDS increased to values comparable to those of NER-proficient cells (Figure 1b).

To further confirm the complementation of DNA repair deficiency by AdXPC infection, XP and proficient DNA repair cells were irradiated with UV light, and cell survival was measured 7 days later (Figure 1c–f). At the doses employed, UV irradiation was not able to generate a significant reduction of survival rates in DNA repair proficient cells (normal human fibroblast, FHN), regardless of previous infection with AdXPC. On the other hand, XP02SP, XP03SP, and XP04SP cells became more resistant to irradiation effects when previously infected with AdXPC. No changes in cell-survival levels were observed in cells infected with AdyXPA (data not shown).

Total RNA and genomic DNA from cell lineages of FHN, XP02SP, XP03SP, and XP04SP were extracted to identify molecular mutations in the *XPC* gene.

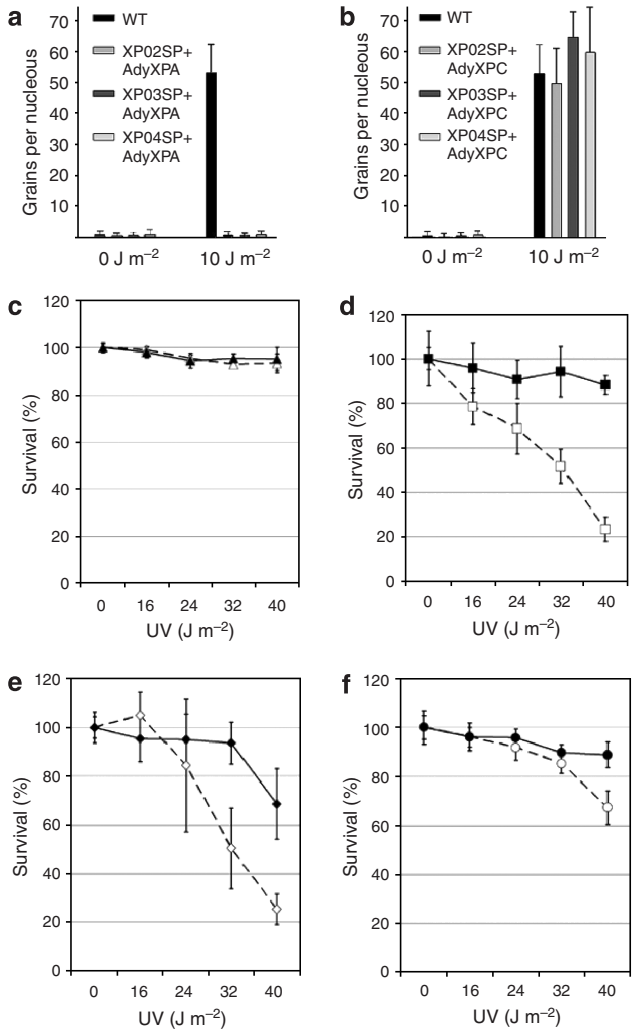

Figure 1. Identification of XP complementation group. Evaluation of NER activity by UDS for the cells from the different patients, after infection with AdyXPA (**a**) or AdXPC (**b**). Only NER-proficient cells, HCB1 and cells XP02SP, XP03SP and XP04SP infected with AdXPC presented grain number incidence after irradiation. The activity of NER is estimated by number of grains in this essay. UV dose is indicated. Survival curves of proficient NER fibroblast FHN (**c**), XP02SP (**d**), XP03SP (**e**) and XP04SP (**f**) XTT was applied to the medium 7 days after UV irradiation in order to determine cells' metabolic activity. Closed symbols with continuous lines correspond to cells infected with AdXPC 24 hours before irradiation; open symbols with discontinuous lines correspond to non infected cells. All the methodology used in this work is presented in the supplementary materials and methods.

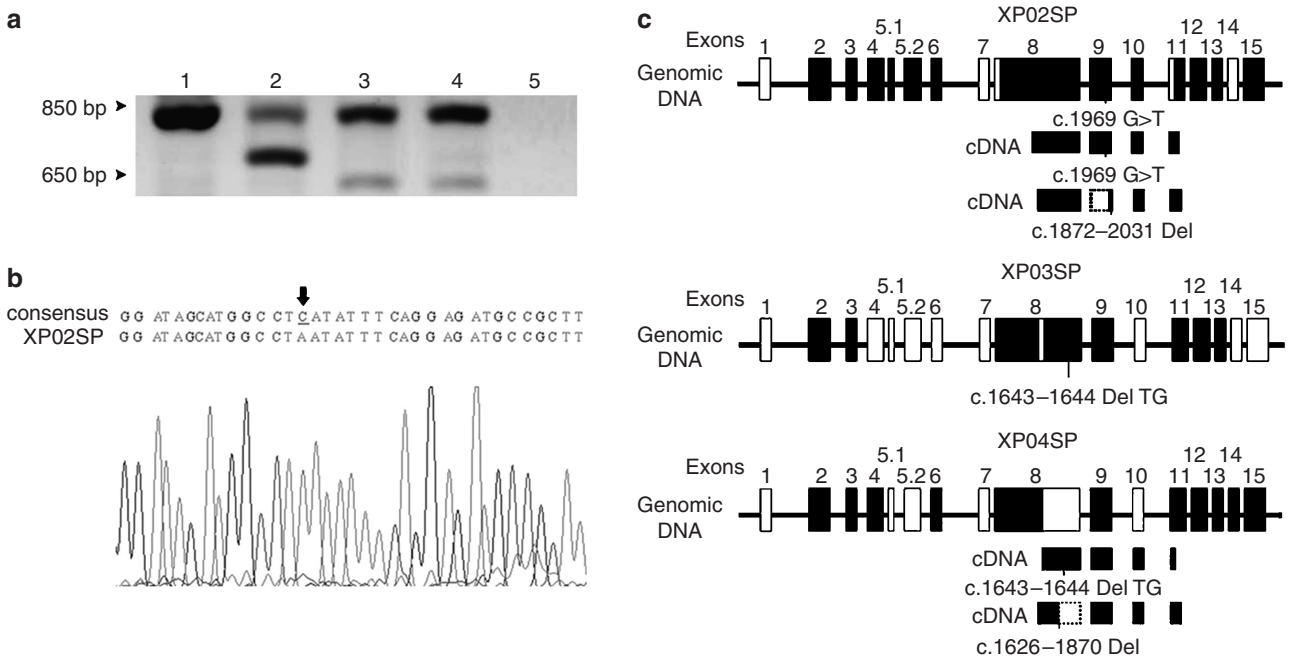

Figure 2. Molecular characterization of *XPC* mutations. (**a**)RT-PCR analysis with primers pair of the region c.1480_2249 of *XPC* from cells FHN (lane 1), XP02SP (lane 2), XP03SP (lane 3), XP04SP (lane 4) and water (lane 5) presenting bands with unexpected sizes in XP cells. (**b**) Alignment of genomic DNA, *XPC* exon 9, indicating a new mutation in c.1969G>T in XP02SP cells. Phrap quality ⩾20. (**c**) Representation of *XPC* sequenced genes and mutations characterized from genomic DNA and cDNA from XP02SP, XP03SP and XP04SP cells. The exons correspond to rectangles and black regions correspond to sequenced areas with quality Phrap ⩾20. Dotted line represents regions absent in cDNA sequences. *XPC* pairs of primers used for the RT-PCR as well as the expected amplicons are shown in Figure S1 and genomic and cDNA primers are presented in Table S1.

Three amplification products resulted in strong and well-defined bands. The amplification of regions c.1_895 (from exon 1 to exon 6) and c.732_1549 (end of exons 5.2 to the first half of the exon 8) result in cDNA fragments with the expected sizes for all cells. However, the product of cDNA amplification for region c.1480_2249 (including the second half of exon 8 to part of exon 11), resulted in bands of the expected sizes (791 bp) for control cells, but different fragments were also observed for the cells from patients (Figure 2a).

The cDNA and genomic DNA from cells were sequenced, and the genetic alterations identified in the *XPC* sequence are described in Table 1. Schematic representation of the mutation observed in one of the XP cells is shown in Figure 2b. Many of the variations correspond to polymorphisms that have already been reported (Table 1).

DNA sequencing for XP02SP cells revealed to our knowledge a previously unreported mutation c.1969G>T (p.Glu657X) at exon 9 for the longer cDNA fragment and for genomic DNA (Figure 2b and c) and a deletion of exon 9 (c.1874_2034) present only at the smaller cDNA amplification fragment (2c). This deletion was not detected in genomic DNA and probably resulted from an mRNA splicing error. The deletion and the mutation potentially generate a truncated protein, with incomplete HR23B and DNA interaction domains and the TFIIH interaction site completely absent (Uchida *et al.*, 2002).

Genomic sequencing revealed a c.1643-1644delTG present at exon 8 in XP03SP cells. This last mutation was confirmed by cDNA sequencing of XP04SP cells (Figure 2c). Another mutation was also observed in the cDNA from XP04SP cells: a deletion of 245 bp of the second half of exon 8 (c.1627_1872del). The two mutations observed for the cDNA on XP03SP and XP04SP potentially generate a frameshift and truncated XPC protein that affects the sites of HR23B, TFIIH interactions, and DNA binding. The c.1643_1644delTG as well as the cDNA deletion (c.1627_1872del) were previously described for XP26PV cells obtained from Italian patients and unrelated kindreds (Chavanne *et al.*, 2000), although the same deletion (c.1627_1872 del) was also described as a result of alternative spliced *XPC* mRNA, from a cryptic splice site, not XP related (Gozukara *et al.*, 2001). The deletion of two bases (c.1643_1644 delTG) was proposed to originate from DNA polymerase slippage at the DNA segment containing a three repeated TG, and configures a hotspot mutation present at the *XPC* gene (Chavanne *et al.*, 2000; Khan *et al.*, 2006).

In summary, this study presents the use of recombinant adenovirus carrying *XP* genes as an efficient tool for diagnosis of XP patients' complementation groups and confirms the molecular characterization of two mutations of the *XPC* gene obtained from Brazilian XP patients, adding to our knowledge a previously unreported mutation causing this syndrome. Besides *XPA* and *XPC* genes, recombinant adenovirus carrying *XPD* and *XPV* genes are also available for complementation analysis (Armelini *et al.*, 2005; Lima-Bessa *et al.*, 2006). We propose the use of these recombinant viruses as an alternative to the other

Table 1. Changes found in cDNA and genomic sequences from FHN, XP02SP, XP03SP, and XP04SP cells (GenBank accession numbers: EU530520–530534)

		Sequencing source				
Cell	Mutation	cDNA	Genomic DNA	Exon	Intron	Comments
FHN						
	c.621+44C>G[1]		x		5.1	Intron variation
	c.1496C>T[2]		x	8		Polymorphism missense p. Ala499Val
	c.2253+43G>C[1]		x		11	Intron variation
	c.2253+77T>G[1]		x		11	Intron variation
XP02SP						
	c.1872_2031del	x		9		Possible splicing error, generates a truncated protein
	c.1969G>T	x	x	9		p.Glu657X, and truncated protein with 657 aa
	c.2253+43G>C[1]		x		11	Intron variation
XP03SP						
	c.1643_1644delTG		x	8		Frameshift and truncated protein with 571 aa
XP04SP						
	c.1643_1644delTG	x		8		Frameshift and truncated protein with 571 aa
	c.1626_1870del	x		8		Possible splicing error, or alternatively spliced XPC mRNA variant
	c.2061A>G[2]	x		10		Polymorphism synonymous p.Arg687Arg

FHN, normal human fibroblast.
[1]GenBank accession numbers: EU530520–530534. These are variations observed in the introns, which have not been described before, although we have found few (two and three) similar sequences at NCBI database. Note that the mutation c.2253+43G>C is observed in both normal and XP02SP cells.
[2]These polymorphisms were previously reported in the NCBI single-nucleotide polymorphisms site (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=7508): rs2228000; rs2227998; rs3731151.

methods used for XP complementation diagnostics, such as somatic cell fusion (Cleaver *et al.*, 1975) and gene reporter after plasmid transfection assays (Carreau *et al.*, 1995; Khan *et al.*, 1998).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Materials and Methods.

Table S1. Sequence of the primers used in this work (*XPC* DNA genomic sequence reference: gi|37550163:14126000-14162000).

Figure S1. Scheme of the *XPC* cDNA indicating the positions of primers used in this work and the expected DNA amplicons. Exon boundaries are indicated by vertical lines. The exon numbers are inside the bar.

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Modulation of Chemotherapy-Induced Human Hair Follicle Damage by 17-β Estradiol and Prednisolone: Potential Stimulators of Normal Hair Regrowth by "Dystrophic Catagen" Promotion?

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TO THE EDITOR

Chemotherapy-induced hair follicle (HF) dystrophy and alopecia are major unresolved problems in clinical oncology (Wang *et al.*, 2006; Bodó *et al.*, 2007; Lemieux *et al.*, 2007). Although the study of cyclophosphamide-induced alopecia in mice has provided important pointers to potentially useful agents that might deserve clinical testing and has allowed us to dissect some of the basic pathobiological mechanisms (Paus *et al.*, 1994, 1996; Schilli *et al.*, 1998; Botchkarev *et al.*, 2000; Ohnemus *et al.*, 2004; Sredni *et al.*, 2004; Hendrix *et al.*, 2005), the—as yet unmet—challenge is to develop a convincing preclinical assay system that allows one to predict how chemotherapy-treated human scalp HFs respond to the various candidate alopecia protectants that have surfaced from rodent studies.

Recently, we established a human *in vitro* model for studying chemotherapy-induced HF dystrophy (Bodó *et al.*, 2007), which reliably re-produces the HF damage seen *in vivo*. In this assay, cyclophosphamide, a classical cytostatic agent that frequently causes chemotherapy-induced alopecia in clinical medicine (Braun-Falco, 1961) is replaced by one of its active toxic metabolites normally generated *in vivo*, 4-hydroperoxycyclophosphamide (4-HC). Using this human model we could show that 4-HC profoundly inhibits hair shaft elongation, matrix keratinocyte proliferation, induces massive apoptosis of matrix keratinocytes and melanin clumping (Bodó *et al.*, 2007).

Chemotherapy-induced HF damage follows two distinct pathways: dystrophic anagen and dystrophic catagen (Paus *et al.*, 1994; Hendrix *et al.*, 2005). During the dystrophic anagen pathway, the hair shaft is shed, and the follicle undergoes an incomplete "primary recovery" during an (paradoxically prolonged!) anagen phase. This is followed by a retarded "secondary recovery" during which a normal hair shaft is generated in the subsequent anagen phase. By contrast, HFs that undergo the dystrophic catagen (DC) pathway (for example, in response to a higher dose of chemotherapy) immediately enter into a dystrophic catagen stage, followed by an abnormally shortened telogen phase, and thus rapidly enter into secondary recovery by premature induction of a new anagen phase. Therefore, even though it is clinically associated with the most dramatic effluvium/alopecia, the DC pathway leads to the fastest, complete HF recovery (Paus *et al.*, 1994; Hendrix *et al.*, 2005).

In mice, *in vivo*, topical application of steroid hormones (dexamethasone, calcitriols, or 17-β-estradiol (E2)) before and after systemic cyclophosphamide administration potently promotes the DC pathway. Thus, these steroid hormones greatly enhance the initial alopecia, but maximally accelerate the regrowth of normally pigmented hair shafts as HF "secondary recovery" is optimally enhanced (Paus *et al.*, 1994; Schilli *et al.*, 1998; Ohnemus *et al.*, 2004).

Therefore, we were interested in studying how E2 or a combination of E2 with glucocorticosteroids would affect 4-HC-induced human scalp HF damage in our new human *in vitro* model (Bodó *et al.*, 2007), and whether there are any indications where these steroid hormones, alone or in combination, promote the DC pathway. We purposely selected E2 and a combination with prednisolone (P) for further study, as the topical application of both has a long tradition of clinical application in central European dermatology for hair loss management (Abadjieva, 2000; Wüstner and Orfanos, 1974). Due to the extremely limited number of available human scalp HFs, we opted for testing of one E2 and P concentration, and selected 10^{-7} M after selected (Kanda and Watanabe, 2004).

Anagen VI HFs were isolated from normal human scalp skin from healthy female patients undergoing routine face-lift surgery (obtained after ethics committee approval and written consent from patient, adhering to Helsinki guidelines) as described (Philpott *et al.*, 1990). We received HFs (10–18 HFs per

Abbreviations: 4-HC, 4-hydroperoxicyclophosphamide; DC, dystrophic catagen pathway; E2, 17-β estradiol; HF, hair follicle; P, prednisolone