Topical Estrogen Accelerates Hair Regrowth in Mice After Chemotherapy-Induced Alopecia by Favoring the Dystrophic Catagen Response Pathway to Damage

Ulrich Ohnemus, Murat Ünalan,* Bori Handjiski,* and Ralf Paus
Department of Dermatology, University Hospital Hamburg-Eppendorf, University of Hamburg, Hamburg, Germany; *Department of Dermatology, Charité, Humboldt-University, Berlin, Germany

Estrogen receptor ligands are important modulators of skin physiology and are involved in the control of normal hair follicle cycling. Here, we have studied the effects of topically applied 17-β-estradiol on pathologic hair follicle cycling as seen during chemotherapy-induced alopecia, one of the major unresolved problems of clinical oncology. For this study we employed a well-established murine model that mimics chemotherapy-induced alopecia in humans. For precisely quantifying the area of hair loss and hair regrowth in this model *in vivo*, we developed a simple planimetric assay (dotmatrix planimetry). We show that topical 17-β-estradiol significantly alters the cycling response of murine follicles to cyclophosphamide, whereas the estrogen antagonist ICI 182.780 exerted no such effects. Initially, topical 17-β-estradiol enhanced chemotherapy-induced alopecia significantly by forcing the follicles into the dystrophic catagen response pathway to hair follicle damage, whereas follicles treated by ICI 182.780 or vehicle shifted into the dystrophic anagen response pathway. Consequently, the regrowth of normally pigmented hair shafts after chemotherapy-induced alopecia was significantly accelerated in the 17-β-estradiol treated group. Our data encourage one to explore topical estrogens as a potential stimulant for hair re-growth after chemotherapy-induced alopecia.

Key words: cyclophosphamide/dotmatrix planimetry/hair follicle dystrophy/hair loss/ICI 182.780.
J Invest Dermatol 122:7–13, 2004

Although there are only few data on the molecular effects of estrogens in human and rodent skin it is now appreciated that estrogens can modulate, after binding to their nuclear receptors (ER-α and ER-β), wound healing, skin aging, cutaneous carcinogenesis, and hair growth (Calvin, 2000; Conrad, *et al*, in press Gendimenico *et al*, 2002; Thornton, 2002). As well as the classical nuclear estrogen signaling pathways, a number of nongenomic 17-β-estradiol (E2) dependent signaling transductions like the mitogen-activated protein kinase cascade are now known to be involved in a variety of biologic processes (Collins and Webb, 1999). Furthermore, in the absence of E2, ER-α and ER-β can be activated through phosphorylation by protein kinase A and C, extracellular signals such as peptide growth factors, cytokines, neurotransmitters, and cell cycle regulators (Chalbos *et al*, 1994; Nilsson *et al*, 2001). With respect to the skin these mechanisms still remain obscure. As for hair growth, it has been shown that the prototypic estrogen E2 inhibits, whereas the ER antagonist ICI 182.780 stimulates hair growth and that gonadectomy results in a profound and rapid telogen to anagen transition. The authors interpreted this E2 effect by an inhibition of the telogen-to-anagen transition of the hair follicle (HF) cycle (Oh and Smart, 1996; Smart *et al*, 1999; Chanda *et al*, 2000; Movérare *et al*, 2002). Species-specific differences in HF responses to estrogens are briefly summarized in Table I.

As the clinically most important human hair growth disorders are based on abnormalities of HF cycling (Paus, 1996; Paus and Cotsarelis, 1999; Stenn and Paus, 2001), we have examined in this study, the effects of exogenous ER ligands on pathologic HF-cycling in mice. Chemotherapy-induced alopecia (CIA) was selected as a model, since it allows analysis of the damage response of the HF and how it is affected by a given test agent (Paus *et al*, 1994, 1996; Slominski *et al*, 1996; Peters *et al*, 2001). In addition, CIA remains one of the most severe problems of clinical oncology that leaves a psychologically disastrous impact on affected patients and their social environment, for which a truly satisfactory remedy remains to be developed in clinical practice (de Vita *et al*, 2001; Bronner and Hood, 1983; Kiebert *et al*, 1990).

Hypothesizing that a systematic analysis of estrogen effects in this model may identify new principles and options for the management of CIA, we used a previously described, well-established mouse model of CIA in C57BL/6 mice with all back skin HF in depilation-induced anagen VI (Paus *et al*, 1994, 1996; Maurer *et al*, 1997; Schilli *et al*, 1998; Peters *et al*, 2001). This model strikingly reproduces the response of the human HF to cyclophosphamide tre-

Abbreviations: CIA, chemotherapy-induced alopecia; E2, 17-β-estradiol; ER, estrogen receptor; ER-α, estrogen receptor type α; ER-β, estrogen receptor type β; HF, hair follicle; ICI 182.780, Imperial Chemical Industries compound no. 182.780 (= pure ER antagonist).

Table I. Species-specific differences of estrogens on hair growth

Species	Reported effect	Reference
Mouse	Spontaneous hair growth is inhibited in pregnant and lactating mice	Fraser and Nay (1953)
Rat	Hair growth waves in female rats lag behind males	Emmens (1942); Johnson (1958)
Guinea pig	Local inhibition of hair growth in guinea pigs receiving topical applications of estradiol	Whitaker (1956); Jackson and Ebling (1972)
Dog	Estrogen inhibits hair growth in dogs	Gardner and De Vita (1940)
Human	Estrogen prolongs the anagen phase of human scalp hair	Schumacher-Stock (1981)

atment under clinical conditions (Braun-Falco, 1961; Paus *et al*, 1994). In order to quantify accurately the area of initial hair loss and subsequent hair regrowth macroscopically and *in vivo*, we developed a simple planimetric assay (dotmatrix planimetry), which invites multiple uses in future hair research (see *Materials and Methods*). HF cycling was assessed by quantitative histomorphometry (Müller-Röver *et al*, 2001). The degree of HF dystrophy, as evident, e.g., from the level and quality of HF pigmentary abnormalities (Paus *et al*, 1994; Slominski *et al*, 1996; Tobin *et al*, 1998), was evaluated using recently defined morphologic guidelines for classifying HF dystrophy (Müller-Röver *et al*, 2002).

In this mouse model of CIA, we had previously established that the HF response to and recovery from chemical damage after cytostatic therapy can be distinguished into the "dystrophic anagen" and the "dystrophic catagen pathway", which determine the onset, the degree, and the quality of the initial alopecia after cytostatic drug administration and the subsequent re-growth of the hair shafts (Paus *et al*, 1994). If the dystrophic anagen pathway is chosen, the damaged HF actually remains longer in anagen than is normally seen, yet produces only functionally impaired, depigmented hair shafts during this recovery within the same anagen phase. If instead, the dystrophic catagen pathway is chosen, anagen is immediately terminated, followed by a dystrophic catagen and a remarkable shortened telogen phase, with the ability to reconstruct a new, fully functional hair shaft factory and HF pigmentary unit (i.e., the anagen hair bulb) at maximal speed. Consequently, HF that choose this damage response pathway, initially, exhibit the most severe and fastest alopecia, yet also have the fastest regrowth of normally pigmented hair shafts during this maximally accelerated secondary recovery phase. Therefore, in addition to assessing the macroscopic effects of these agents on CIA and hair shaft regrowth in this model, we wished to analyze specifically, whether treatment of cyclophosphamide-damaged HF with E2 or the ER antagonist ICI 182.720 favored the dystrophic anagen or the dystrophic catagen response pathway to chemical HF damage.

Results and Discussion

Topical E2 accelerates the development of alopecia after chemotherapy Initially, we evaluated how exogenous, topically applied E2 influences the development of alopecia after chemotherapy. Five days after CIA induction (i.e., 14 d after anagen induction by depilation), mice that had been treated with the vehicle alone or with the ER antagonist ICI 182.780 showed marked anagen effluvium with circular or irregularly shaped alopecic areas. In contrast, E2-treated animals exhibited large and confluent areas of complete baldness (Fig 1), and the area of alopecic back skin was significantly larger ($p \leqslant 0.001$) in E2-treated animals than in the other groups (Fig 2). On day 16 after anagen induction there were no macroscopic differences between the three groups and all animals displayed complete alopecia. A time course is given in Fig 3.

E2 promotes the dystrophic catagen HF response pathway to damage Quantitative histomorphometry revealed that E2-treated animals predominantly showed HF in dystrophic catagen stages (Fig 4). These were characterized by shortened, catagen-like HF and abnormally dilated hair canals that did not contain hair shafts, along with pigmentary abnormalities such as melanin incontinence and clumping of melanosomes. In contrast to the E2-treated group, vehicle or ICI 182.780-treated mice exhibited predominantly dystrophic anagen follicles 14 d after depilation (Fig 4). These were characterized by clumped melanin globules, and a reduction in size of the dermal papilla below one-third of the hair bulb diameter. Figure 5(*A*) illustrates E2-treated follicles that are mainly in mid-dystrophic catagen, whereas Fig 5(*B*) shows E2 treated, mid to late dystrophic catagen follicles, 14 d after depilation. As seen in Fig 5(*C,D*), vehicle-treated follicles display strong to severe dystrophic anagen, following recently defined guidelines for assessing the degree of HF dystrophy (Müller-Röver *et al*, 2002). Therefore, E2 has a catagen-promoting effect and interacts with the two defined pathways of HF response to chemical damage by strongly favoring the dystrophic catagen over the dystrophic anagen pathway (Fig 6) (Paus *et al*, 1994). The catagen-promoting properties of E2 seen in this CIA model is perfectly in line with our recent identification of topical E2 as a potent catagen inducer. In a separate study we observed that exogenous applied E2, if compared with ICI 182.780 or control, significantly accelerated catagen development in anagen HF during normal murine hair cycling. Between the control and the ICI 182.780-treated group no significant differences were observed[1].

[1]Ohnemus U, Unalan M, Handjiski B, Nakamura M, Liotiri S, Conrad F, Bettermann A, Paus R. Estrogen effects on murine and human hair follicle cycling. JID 121:824, 2003

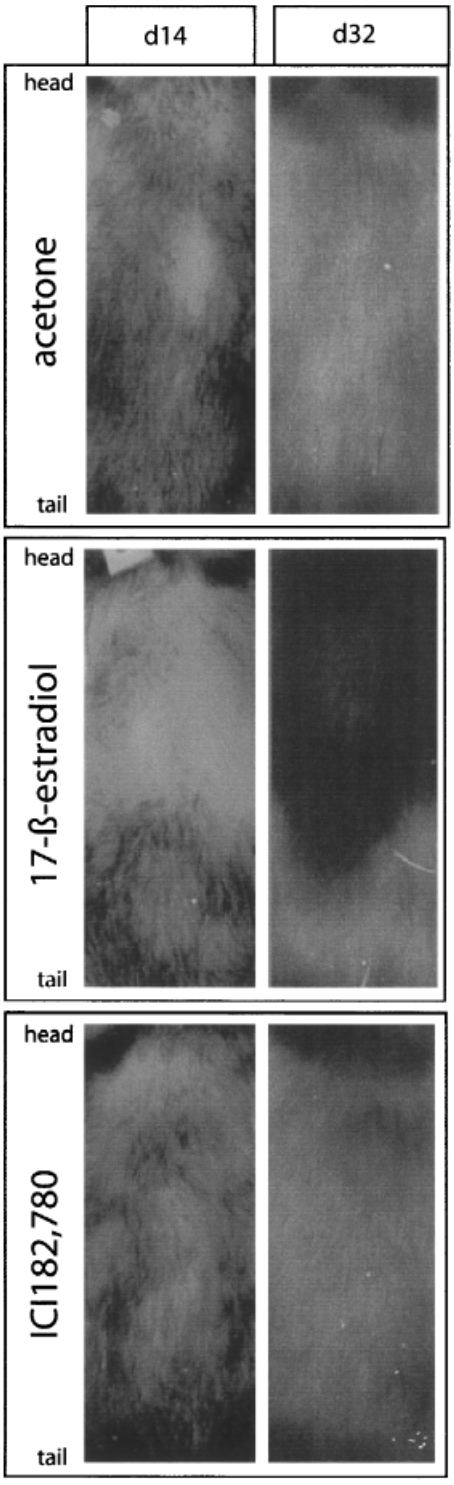

Figure 1
Macroscopic effects (alopecia and skin color changes) after CYP injection in depilated murine back skin. Representative mice backs are shown. Left column: day 14 after depilation (5 d after CYP injection). Note that mice treated with E2 show big areas of bald back skin and in areas without maximal alopecia a translucent, grayish skin color. Vehicle or ICI 182.780-treated animals predominantly exert isolated and small circular areas with complete baldness. Right column: day 32 after depilation (23 d after CYP injection). Follicles have entered a new cycle with a synchronized wave of anagen follicles (secondary recovery). E2-treated animals show full recovery from CIA with enhanced and better pigmented hair regrowth compared with the control and the ICI 182.780-treated group. The figure also demonstrates that alopecia was more severe in the neck than in the tail area of murine back skin.

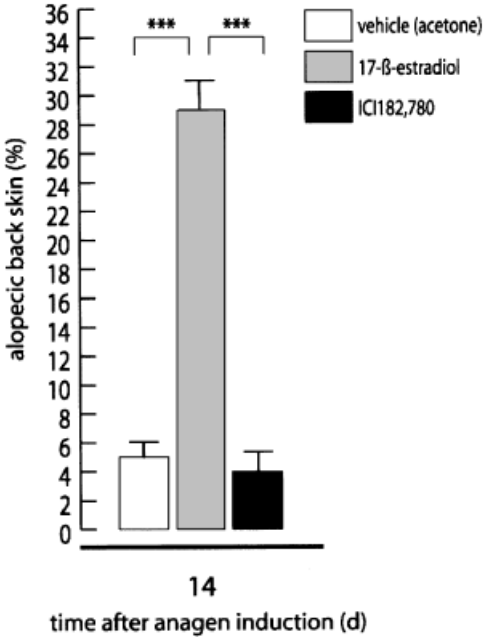

Figure 2
Dotmatrix analysis of CIA in C57BL/6 mice on day 14 after depilation. CYP injection 120 mg per kg on day 9 after anagen induction. The graph shows the percentage of animals with alopecic back skin. E2 treatment promotes significant enhancement of CYP-induced alopecia, whereas ICI 182.780 fails to protect from it. Given are the mean $\pm$ 1 SEM of pooled data from three identical experiments, $n = 90$, representing a total of 30 animals per group. Level of significance compared with control: $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$; $^{***}p \leqslant 0.001$.

The estrogen receptor antagonist ICI 182.780 failed to protect from alopecia and the animals showed the same extent of CIA and HF dystrophy as the vehicle-treated controls (Fig 4). E2 shares this property with other nuclear hormone receptor ligands, such as dexamethasone and calcitriol (Paus *et al*, 1996; Schilli *et al*, 1998) as well as with PTH/PTHPrP receptor agonists (Peters *et al*, 2001), all of which have in common the fact that they fail to protect from CIA in this mouse model and, moreover, accelerate pathologic cyclophosphamide-induced catagen, thus worsening the initial alopecia after cyclophosphamide injection (Paus *et al*, 1994). This further supports our previous postulate that catagen-promoting substances also promote the dystrophic catagen pathway whenever the HF is damaged (Paus *et al*, 1994). As the degree of dystrophic catagen development during CIA is proportional to the cyclophosphamide dose employed and therefore to the degree of chemical damage as well as to the amount of apoptosis induced as a consequence, one may speculate that both apoptosis-driven events (catagen, CIA) promote each other (Paus *et al*, 1994, 1996; Schilli *et al*, 1998).

Topical E2 accelerates hair re-growth after chemotherapy Based on our previous findings (Fig 6), one would expect that promotion of the dystrophic catagen pathway also promotes HF recovery and the regrowth of fully functional, normally pigmented hair shafts. Therefore, the chemotherapy experiment was extended and the influence of E2 on the recovery period was investigated, 28 to 32 d after chemotherapy. Vehicle- or ICI 182.780-treated animals showed only sparse hair regrowth. About 25 to 30% of the

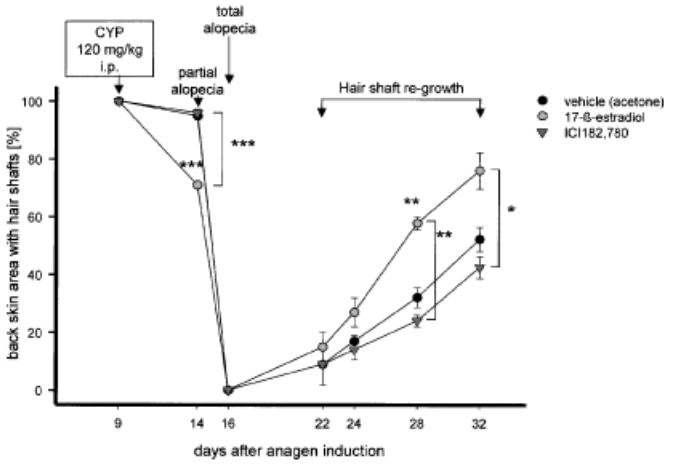

Figure 3
Time course of visible alopecia and hair re-growth from day 9 until day 32 after anagen induction. The graph shows the percentage of back skin areas with hair shafts over the time from day 9 until day 32 after anagen induction, assessed by dotmatrix planimetry. It demonstrates that E2 significantly accelerates the course of alopecia induced by CYP injection from day 9 until day 16 compared with control or ICI 182.780-treated animals. From day 22 until day 32 hair shaft regrowth is accelerated under E2 treatment, compared with vehicle and ICI 182.780-treated animals. There are significant differences between animals treated with ICI 182.780 and E2, as well as between control and E2-treated animals on day 28. On day 32 the differences are only significant between ICI 182.780- and E2-treated animals. Given are the mean $\pm$ 1 SEM of pooled data from three identical experiments. Day 9: $n = 129$, representing a total of 43 animals per group. Day 14: $n = 90$, representing a total of 30 animals per group. Day 16, 22, 24, 28, and 32: $n = 51$, representing a total of 17 animals per group. Level of significance compared with control: $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$; $^{***}p \leqslant 0.001$.

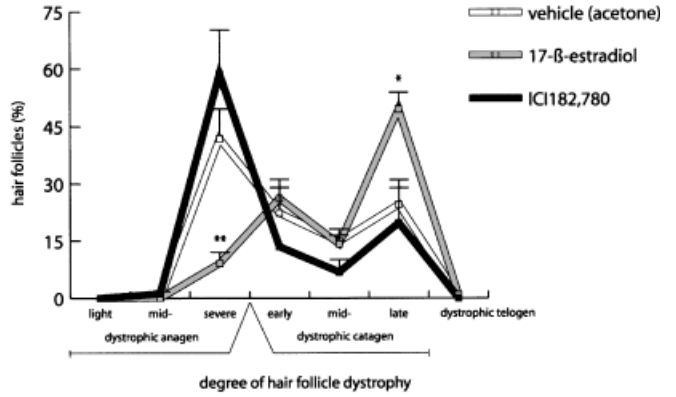

Figure 4
Morphometric analysis of CYP-induced dystrophic anagen *versus* dystrophic catagen on day 14 postdepilation, i.e., 5 d after single dose of 120 mg CYP per kg. None of the compounds protects from HF dystrophy caused by chemotherapy. Compared with the control and the ICI 182.780-treated groups, mice treated with E2 display significantly more follicles in dystrophic catagen and significantly less follicles in dystrophic anagen. Given are the mean $\pm$ 1 SEM, $n = 39$, representing a total of 13 animals per group. Level of significance compared with control: $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$.

back skin area was markedly pigmented, indicating the regrowth of pigmented hair shafts during the subsequent anagen phase (Figs 1 and 7). In contrast, E2-treated animals revealed a significantly larger area of hair regrowth (Figs 1 and 7). The time course of hair shaft regrowth is given in Fig 3. Histomorphometry confirmed that E2-treated follicles had already entered into secondary recovery by showing a

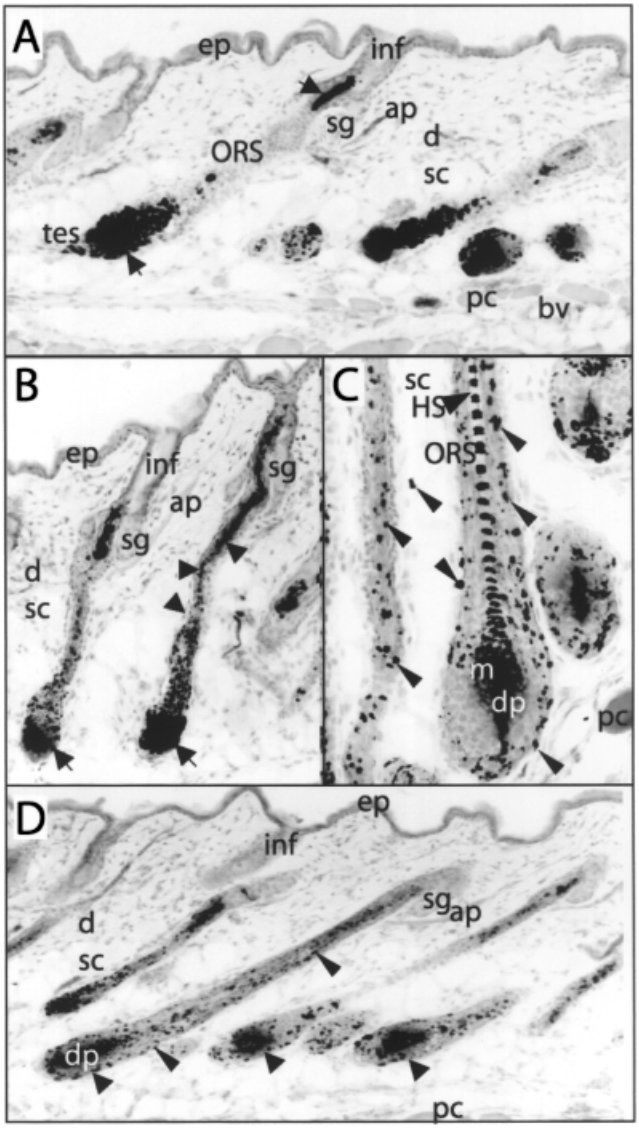

Figure 5
Representative histopathology on day 14 after depilation, i.e., 5 d after CYP injection. Sections impressively display pigmentation disorders, which were induced by CYP. (*A*,*B*) E2-treated group; (*C,D*) vehicle control. (*A,B)* Show mid-to-late dystrophic catagen follicles as mainly seen in the E2-treated CIA skin. *Arrows* indicate clumping of melanin and abnormally dilated hair canals not containing a hair shaft. Ectopic distribution of melanin as a sign of dystrophy can be seen in (*C*). (*C,D*) Sections of severe dystrophic anagen follicles, typical for the vehicle-treated control and the ICI 182.780-treated group. ap, arrector pili muscle; bv, blood vessel; d, dermis; dp, dermal papilla; ep, epithelium; HS, hair shaft; inf, infundibulum; m, hair matrix; ORS, outer root sheath; pc, panniculus carnosus muscle; sc, subcutis; sg, sebaceous gland; tes, tailing epithelial strand.

predominance of anagen VI HF, whereas the two other groups exhibited large percentages of late catagen and early anagen stages (Fig 8). Morphologically the reconstructed anagen VI HF of E2-treated mice were of normal appearance, with only a few ectopic melanin granula visible in the dermis, as a morphologic record of the damage the preceding anagen HF and its pigmentary unit had suffered (Fig 8). During the observation time neither significant alterations or advances of hair regrowth nor improvement in the regrowth of well-pigmented hair shafts after CIA were apparent under ICI 182.780 treatment (Fig 8).

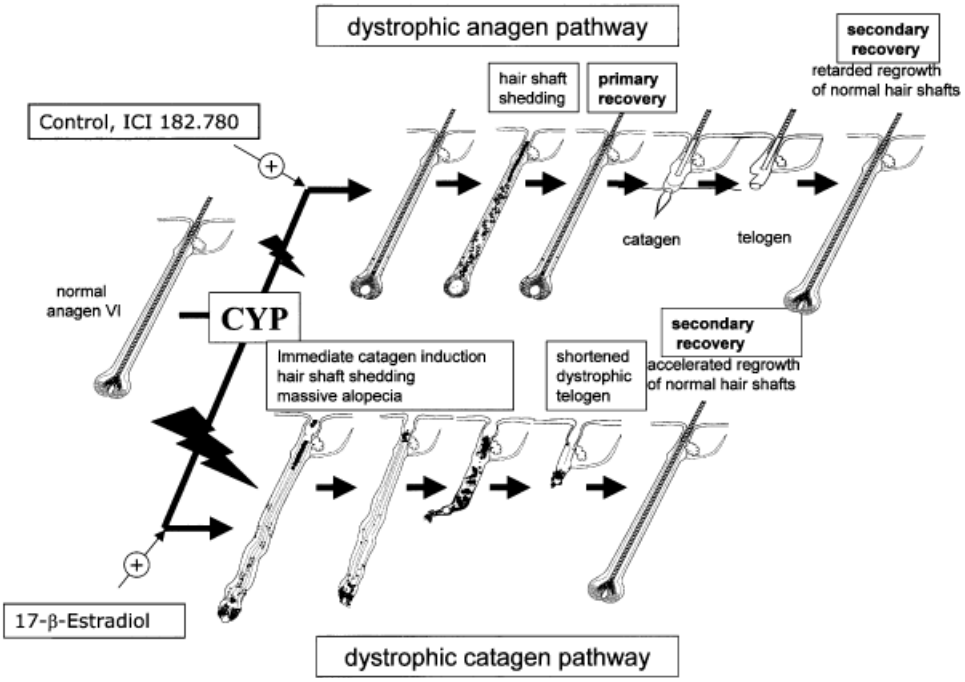

Figure 6
Dystrophic pathways (anagen and catagen) of CIA. Schematic representation of the two different pathways of CYP-induced HF dystrophy: dystrophic anagen and dystrophic catagen pathway (dysA and dysC). Size of the black flashes indicate the degree of follicle damage. Vehicle control (acetone) and ICI 182.780 promote the dysA pathway, E2 promotes the dysC pathway. During the dysA pathway the hair shaft is shed and the follicle undergoes a primary recovery. The follicle is not fully recovered before it undergoes a complete catagen–telogen transition to enter the final secondary recovery stage. During the dysC pathway, follicles are severely damaged and enter directly into dysC and a shortened dystrophic telogen. Without passing through the primary recovery the follicles enter directly into the complete secondary recovery stage with normal pigmented hair shafts (modified after Muller-Rover *et al*, 2002).

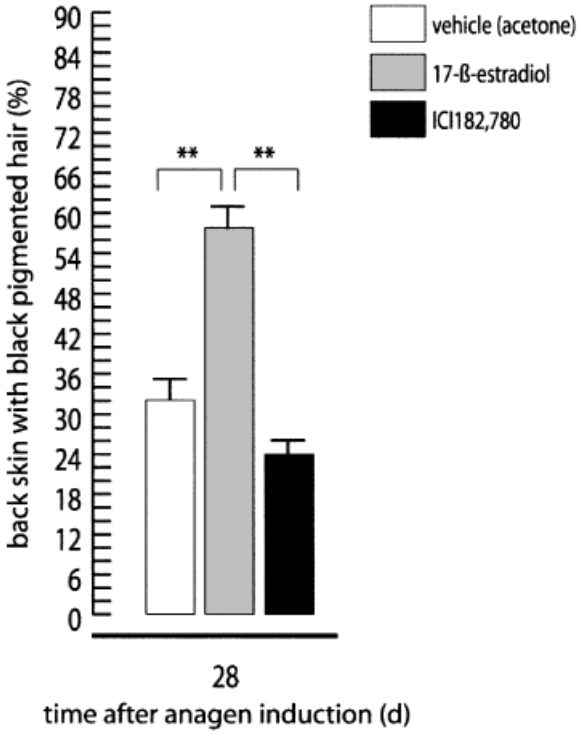

Figure 7
Dotmatrix analysis of recovery from CIA and regrowth of normal pigmented hair shafts 28 d after anagen induction, i.e., 19 d after CYP injection. The panel shows that E2 significantly accelerates recovery from CIA and regrowth of normal pigmented hair shafts compared with control or ICI 182.780-treated animals. Given are the mean $\pm$ 1 SEM, n = 51, representing a total of 17 animals per group. Level of significance compared with control: $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$.

This supports the concept that follicles that respond to chemotherapy by entering dystrophic anagen retain their hair shafts longer but have greater difficulties in recovering from chemical damage than follicles entering dystrophic catagen, and therefore promoting secondary recovery (Fig 6) (Paus *et al*, 1994, 1996; Schilli *et al*, 1998; Peters *et al*, 2001). The latter rapidly run through a dystrophic catagen and a dramatically shortened telogen phase, and produce new hair shafts only during the following hair cycle. This secondary recovery phase occurs much earlier than in dystrophic anagen HF and generates mainly healthier, well-pigmented hair shafts (Fig 6). A previous report that topical E2 arrests normal murine back skin HF in the telogen stage of the hair cycle and that only telogen HF express ER (Oh and Smart, 1996), would have suggested that E2 also significantly retards the re-growth of normal anagen HF from dystrophic catagen HF. The fact that the opposite was seen suggests, however, that in contrast to previously voiced concepts (Oh and Smart, 1996; Smart *et al*, 1999) anagen VI HF are quite responsive to ER stimulation and are thereby induced to enter catagen prematurely. This is in line with our recent finding that E2 is indeed a potent catagen inducer in normal murine HF and that late anagen VI and catagen HF in C57BL/6 mice do express ER-α and ER-β on the gene and protein level.[1]

Although the effects exerted by E2 observed in the current CIA model resemble those of other steroid hormones, namely glucocorticosteroids (Paus *et al*, 1994) and calcitriol (Paus *et al*, 1996; Schilli *et al*, 1998), the underlying mechanisms which they are controlled remain to be elucidated. The complex molecular signaling pathways of the two estrogen receptors ER α and ER β, their intricate interactions with agonists and antagonists, multiple signal cross-talk mechanisms, and a plethora of molecules involved in ER signaling (coactivation, corepression) (Paech *et al*, 1997; Nilsson and Gustafsson, 2000; Nilsson *et al*, 2001; Thornton, 2002), make it a dauntingly difficult task to determine which cellular responses to ER ligands within the HF or its direct vicinity are responsible for the observed hair cycle-modulatory effects of E2. Our data, however, suggest that topically applied ER agonists could become an effective tool in the control of pathologic HF cycling and

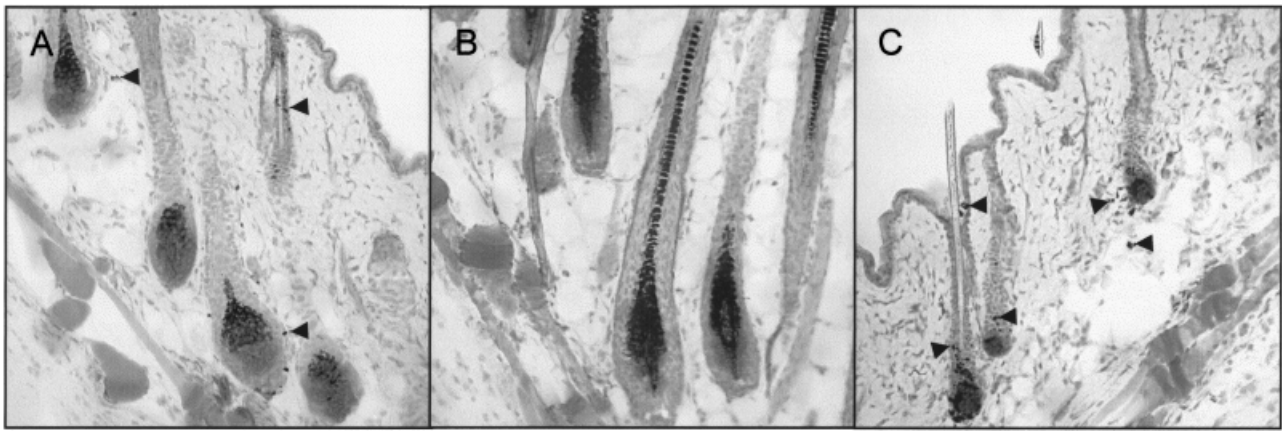

Figure 8
Representative histopathology on day 32 after depilation, i.e., 23 d after CYP injection. (*A,C*) HF that had undergone the dystrophic anagen pathway; (*B*) follicles that had undergone the dystrophic catagen pathway. On day 32 the control-treated groups (*A*) and ICI-treated groups (*C*) are predominately in anagen stage IV (*A*) and stage II (*C*). The *arrowheads* indicate ectopic melanin granules as signs of previous dystrophy. (*B*) Follicles from the E2-treated group that are mainly in reconstructed anagen stage VI with normal appearance and no signs of dystrophy.

be used, whenever desired, for premature catagen induction. Additionally, estrogens deserve to be fully explored as potential stimulators of hair regrowth after CIA in humans.

Materials and Methods

Animals Six to 9 wk old syngenic female C57BL/6 mice (15–20g) were purchased from Charles River (Sulzfeld, Germany) and housed in community cages with 12 h light periods at the Charité Animal Facilities, Campus Virchow Hospital. They were fed water and mouse chow *ad libitum*. All experiments were performed in accordance with applicable German laws on the use of laboratory animals.

CIA model and E2 or ICI 182.780 treatment Mice with all back skin HF in telogen, as recognized by their pink skin color, were induced to enter active hair growth (anagen) by hair shaft depilation, as described in detail before (Paus *et al*, 1990). CIA was generated as previously described (Paus *et al*, 1994). Briefly, in three identical, independent experiments with a total of 86 test and 43 control animals three groups of mice received intraperitoneally a single dose of 120 mg per kg cyclophosphamide (ENDOXAN, ASTA-Medica, Frankfurt, Germany) in 0.9% NaCl 9 d after depilation. Starting 1 d before the cyclophosphamide injection, mice were treated topically with 10 nmol E2 or with ICI 182.780 (E2 from Schering AG, Berlin, Germany, gift of Dr A. Menrad, ICI 182.780 from ASTRA ZENECA, Wedel, Germany) once daily for 5 d. Acetone, used as vehicle, was applied as the control. Individual mice from the test and control groups were euthanized randomly for histology at selected time points. On day 32, all remaining mice were euthanized and back skin was embedded as described previously (Paus *et al*, 1999). Macroscopic and microscopic analyses of the test and control mice were performed between day 10 and 32 after anagen induction.

Macroscopic analysis by ''dotmatrix planimetry'' For the accurate, quantitative assessment of cyclophosphamide-induced alopecia and hair regrowth a new planimetric assay (dotmatrix planimetry) was developed. Under a ketamine anesthesia, mouse back skin is clipped with electric clippers and photos are taken from the back skin. A transparency (e.g., for overhead projectors) is placed on each developed photo and the appropriate areas (e.g., of hair loss or hair regrowth) are demarcated on the foil. Afterwards a printed sheet with a defined, uniform dot pattern (i.e., the dotmatrix) is placed under the transparent foil to quantify the percentage of the marked area relative to the total reference area, and is examined under a desk monitor-projector combination (Reflecta Diamantor AFM, MC Germany). The encircled underlying dots are counted and related to the dot count of the whole back skin area. Measuring areas by counting dots is superior to other possible methods due to the nongeometrically, irregularly shaped, encircled skin areas.

Microscopic analysis by quantitative histomorphometry After cyclophosphamide injection, quantitative histomorphometry was performed at different time points, i.e., HF were classified according to their hair cycle stage and signs of follicle dystrophy (Paus *et al*, 1994, 1996; Tobin *et al*, 1998; Müller-Röver *et al*, 2001, 2002). The time points of skin harvesting and the number of animals analyzed for performing histomorphometry are given in the figure legends. Morphometry was performed on Giemsa-stained 8 μm formalin-fixed, paraffin-embedded sections, which were taken from defined back skin regions. A minimum of 50 back skin HF were analyzed for each test and control mouse.

Statistical analysis Data derived from identical experiments were pooled and mean values were calculated. With the statistical analysis software SPSS (SPSS Inc., Chicago, Illinois), significance was assessed with the Mann–Whitney U test for unpaired samples combined with Monte-Carlo calculation for accuracy. $p \leqslant 0.05$ were accepted as significant.

The authors are grateful to Ruth Pliet for excellent technical assistance, and to Dr Andreas Menrad for support and advice. This study was supported in part by grants from Schering AG, Berlin, and Cutech Srl., Padova.

DOI: 10.1046/j.0022-202X.2003.22120.x

Manuscript received May 22, 2003; revised September 3, 2003; accepted for publication September 23, 2003

Address correspondence to: Ralf Paus, MD, Department of Dermatology, University Hospital Hamburg-Eppendorf, University of Hamburg, Martinistr. 52, D-20246 Hamburg, Germany. Email: paus@uke.uni-hamburg.de

References

Braun-Falco O: Klinik und Pathomechanismus der Endoxan-Alopecie als Beitrag zum Wesen cytostatischer Alopecien. Arch Klin Exp Dermatol 212:194–216, 1961

Bronner AK, Hood AF: Cutaneous complications of chemotherapeutic agents. J Am Acad Dermatol 9:645–663, 1983

Calvin M: Oestrogens and wound healing. Maturitas 34:195–210, 2000

Chalbos D, Philips A, Rochefort H: Genomic cross-talk between the estrogen receptor and growth factor regulatory pathways in estrogen target tissues. Semin Cancer Biol 5:361–368, 1994

Chanda S, Robinette CL, Couse JF, Smart RC: 17beta-estradiol and ICI-182780 regulate the hair follicle cycle in mice through an estrogen receptor-alpha pathway. Am J Physiol Endocrinol Metab 278:E202–E210, 2000

Collins P, Webb C: Estrogen hits the surface. Nature Med 5:1130–1131, 1999

Corad F, Ohnermus M, Bodo E, Bettermann A, Paus R: Estrogens and human scalp hair growth: Still more questions than answers. J Invest Dermatol: in press

Emmens CW: The endocrine system and hair growth in the rat. J Endocrinol 3:64–78, 1942

Fraser AS, Nay T: Growth of the mouse coat II. Effects of sex and pregnancy. Aust J Biol Sci 6:645–656, 1953

Gardner WK, De Vita J: Inhibition of hair growth in dogs receiving estrogens. Yale J Biol Med 13:213–216, 1940

Gendimenico GJ, Mack VJ, Siock PA, Mezick JA: Topical estrogens: Their effects on connective tissue synthesis in hairless mouse skin. *Arch Dermatol Res* 294:231–236, 2002

Jackson D, Ebling FJ: The activity of hair follicles and their response to oestradiol in the guinea-pig *Cavia porcellus* L. J Anat 111:303–316, 1972

Johnson E: Quantitative studies of hair growth in the albino rat. II. The effect of sex hormones. J Endocrinol 16:351–359, 1958

Kiebert GM, Hanneke J, de Haes CJ, Kievit J, van de Velde CJ: Effect of perioperative chemotharapy on quality of life of patients with early breast cancer. Eur J Cancer 26:1038–1042, 1990

Maurer M, Handjiski B, Paus R: Hair growth modulation by topical immunophilin ligands: Induction of anagen, inhibition of massive catagen development, and relative protection from chemotherapy-induced alopecia. Am J Pathol 150:1433–1441, 1997

Movérare S, Lindberg MK, Faergemann J, Gustafsson J-A, Ohlsson C: Estrogen receptor α, but not estrogen receptor β, is involved in the regulation of the hair follicle cycling as well as the thickness of epidermis in male mice. J Invest Dermatol 119:1053–1058, 2002

Müller-Röver S, Handjiski B, van der Veen C, *et al*: A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. J Invest Dermatol 117:3–15, 2001

Müller-Röver S, Handjiski B, Paus R: Pathways of chemotherapy-induced alopecia: New criteria for the diagnosis of hair follicle dystrophy. Arch Dermatol Res 294:40, 2002

Nilsson S, Gustafsson J-A: Estrogen receptor transcription and transactivation. Basic aspects of estrogen action. Cancer Res 2:360–366, 2000

Nilsson S, Makela S, Treuter E, *et al*: Mechanisms of estrogen action. Physiol Rev 81:1535–1565, 2001

Oh HS, Smart RC: An estrogen receptor pathway regulates the telogen-anagen hair follicle transition and influences epidermal cell proliferation. Proc Natl Acad Sci USA 93:12525–12530, 1996

Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J-A, Kushner PJ, Scanlan TS: Differential ligand activation of estrogen receptors ER α and ER β at AP 1 sites. Science 277:1508–1510, 1997

Paus R: Control of the hair cycle and hair diseases as cycling disorders. Curr Opin Dermatol 3:248–258, 1996

Paus R, Cotsarelis G: The biology of hair follicles. N Engl J Med 341:491–497, 1999

Paus R, Stenn KS, Link RE: Telogen skin contains an inhibitor of hair growth. Br J Dermatol 122:777–784, 1990

Paus R, Handjiski B, Eichmuller S, Czarnetzki B: Chemotherapy-induced alopecia in mice. Induction by cyclophosphamide, inhibition by cyclosporine A, and modulation by dexamethasone. Am J Pathol 144:719–734, 1994

Paus R, Schilli MB, Handjiski B, Menrad A, Henz B, Plonka P: Topical calcitriol enhances normal hair regrowth but does not prevent chemotherapy-induced alopecia in mice. Cancer Res 56:4438–4443, 1996

Paus R, Müller-Röver S, Van der Veen C, *et al*: A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis. J Invest Dermatol 113:523–532, 1999

Peters EM, Foitzik K, Paus R, Ray S, Holick M: A new strategy for modulating chemotherapy-induced alopecia, using PTH/PTHrP receptor agonist and antagonist. J Invest Dermatol 117:173–178, 2001

Schilli MB, Paus R, Menrad A: Reduction of intrafollicular apoptosis in chemotherapy-induced alopecia by topical calcitriol-analogs. J Invest Dermatol 111:598–604, 1998

Schumacher-Stock U: Estrogen treatment of hair diseases. In Orfanos CE, Montagna W, Stuttgen G: (eds). Hair Research. Berlin: Springer-Verlag, 1981; p 318–332

Slominski A, Paus R, Plonka P, Handjiski B, Maurer M, Chakraborty A, Mihm MC Jr: Pharmacological disruption of hair follicle pigmentation by cyclophosphamide as a model for studying the melanocyte response to and recovery from cytotoxic drug damage in situ. J Invest Dermatol 106: 1203–1211, 1996

Smart RC, Oh HS, Chanda S, Robinette CL: Effects of 17-β-estradiol and ICl 182 780 on hair growth in various strains of mice. J Investig Dermatol Symp Proc 4:285–289, 1999

Stenn KS, Paus R: Controls of hair follicle cycling. Physiol Rev 81:449–494, 2001

Thornton MJ: The biological actions of estrogens on skin. Exp Dermatol 11:487–502, 2002

Tobin DJ, Hagen E, Botchkarev VA, Paus R: Do hair bulb melanocytes undergo apoptosis during hair follicle regression (catagen)? J Invest Dermatol 111:941–947, 1998

de Vita V, Hellman S, Rosenberg S (eds.): Cancer, Principles and Practice of Oncology. Philadelphia: Lippincott, Williams and Willikins, 2001

Whitaker WL: Local inhibition of hair growth in the guinea pig by the topical application of α-estradiol. Anat Record 124:447–448, 1958