A Guide to Assessing Damage Response Pathways of the Hair Follicle: Lessons From Cyclophosphamide-Induced Alopecia in Mice

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After chemical, biological, or physical damage, growing (i.e. anagen) hair follicles develop abnormalities that are collectively called *hair follicle dystrophy*. Comparatively lower follicular damage induces the ''dystrophic anagen'' response pathway (=prolonged, dystrophic anagen, followed by severely retarded follicular recovery). More severe follicular damage induces the dystrophic catagen pathway (=immediate anagen termination, followed by a dystrophic, abnormally shortened telogen and maximally fast follicular recovery). In order to recognize these distinct damage response strategies of the hair follicle in a clinical or histopathological context, we have used the well-established C57BL/6J mouse model of cyclophosphamide-induced alopecia to define pragmatic classification criteria for hair follicle dystrophy (e.g., structure and pigmentation of the hair shaft, location, and volume of ectopic melanin granules, distension of follicular canal, number of TdT-mediated dUTP nick end labeling positive keratinocytes in the hair bulb; neural cell-adhesion molecule immunoreactivity and alkaline phosphatase activity as markers for the level of damage to the follicular papilla). These classification criteria for hair follicle dystrophy are useful not only in chemotherapy-induced alopecia models, but also in the screening of drug-treated or mutant mice in a highly standardized, accurate, sensitive, reproducible, easily applicable, and quantifiable manner.

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In response to damage, hair follicles undergo two distinct pathways of dystrophy, which are characterized by specific morphological abnormalities. These are particularly prominent and clinically highly relevant in the course of chemotherapy-induced alopecia (CIA). Decades ago, different types of alopecias as well as some key parameters for the recognition of defined stages of chemotherapy-induced hair follicle dystrophy in mammals were defined (Braun-Falco and Theisen, 1959; Braun-Falco, 1961, 1966; Zaun, 1964; Herzberg, 1966; Kostanecki *et al*, 1966; Homan *et al*, 1968). For nearly 40 y, these publications have been the only references for the classification of hair follicle dystrophy, although none of them offers a comprehensive, unified classification scheme for use in the laboratory. For this reason, we have developed a set of pragmatic classification criteria for hair follicle dystrophy, using the C57BL/6J mouse model of cyclophosphamide (CYP)-induced alopecia (Fig 1) (Paus *et al*, 1994c, 1996; Slominski *et al*, 1996; Schilli *et al*, 1998; Müller-Röver *et al*, 2000; Peters *et al*, 2001). Studying this model, we had found that hair follicles undergo two distinct pathways of dystrophy when they have suffered chemical damage (here: by cytostatic drugs) (Paus *et al*, 1994c). These two damage-response pathways are characterized by specific morphological abnormalities, which eventually lead to hair loss and alopecia (Fig 2). Therefore, guidelines for the accurate and standardized classification of chemotherapy-induced hair follicle dystrophy are urgently needed, not the least in order to assist in the ongoing quest to combat CIA by the development of more effective alopecia-protection strategies.

This review complements our earlier guides on the classification of murine hair follicle development (Paus *et al*, 1999) and hair follicle cycling (Müller-Röver *et al*, 2001) so as to provide a standardized approach to the analysis of murine hair follicle dystrophy. It serves as a useful companion to a similar guide that has recently been published for the assessment of human hair follicle dystrophy (Whiting, 2003), and should be of particular interest to all researchers that wish to professionally assess hair follicle damage inflicted by test agents, engineered or spontaneous mutations and a wide range of diseases. Given the exquisite sensitivity of the hair follicle as a "biological damage indicator" that is negatively affected, e.g. by an enormous number of different clinically widely used drugs (cf. Litt, 2004) and by numerous mutations (Nakamura *et al*, 2002)., a professional assessment of hair follicle dystrophy along the lines indicated here exemplarily for CIA offers a simple, yet reliable comprehensive and instructive tool for

Abbreviations: AP, alkaline phosphatase; CIA, chemotherapy-induced alopecia; CTS, connective tissue sheath; CYP, cyclophosphamide; DP, dermal papilla; IRS, inner root sheath; NCAM, neural cell-adhesion molecule; ORS, outer root sheath; TUNEL, TdT-mediated dUTP nick end labeling

obtaining novel insights into the biological effects of test agents.

Based on basic histological and ultrastructural studies on human and rodent CIA (Braun-Falco, 1966; Herzberg, 1966), we have summarized basic as well as more advanced auxiliary criteria to define the distinct stages of the dystrophic anagen and the dystrophic catagen pathway (Figs 3 and 4) which are widely applicable to different mouse strains and mutants. In essence, this classification guide can also be utilized for staging the hair follicles of other hair-bearing animals, even though species-specific anatomic differences must be taken into account.

Hair follicle cycling in young mice follows a rather precise timescale (Paus *et al*, 1999; Müller-Röver *et al*, 2001). Nevertheless, the fine details of hair follicle cycling are dependent on the genetic background (mouse strain), the sex (e.g., female mice show a prolonged telogen; S. Müller-Röver, unpublished observation) as well as environmental factors such as time of the year (temperature, light periods) and nutritional factors. To avoid associated fluctuations, the present guide is based on the most extensively studied and best standardized hair research model, the C57BL/6J models of depilation-induced hair cycling (Chase, 1954; Paus *et al*, 1990, 1994a, b; Müller-Röver *et al*, 2001) and CYP-induced alopecia (Paus *et al*, 1994c, 1996; Slominski *et al*, 1996; Schilli *et al*, 1998; Müller-Röver *et al*, 2000; Peters *et al*, 2001; Ohnemus *et al*, 2004). Briefly, a wax/rosin mixture is applied on the dorsal skin of 7-wk-old mice with all dorsal skin hair follicles in the resting phase (telogen), as evidenced by the homogeneously pink back skin color. Plucking of the wax/rosin mixture removes all hair shafts and immediately induces anagen development of unparalleled homogeneity and synchrony over the entire depilated back of the mouse (Chase, 1954; Müller-Röver *et al*, 2001). Nine days after depilation, all depilated hair follicles have entered the final stage of the growth phase of the hair cycle (anagen VI). Around day 17 after depilation the follicles spontaneously start to undergo regression (catagen) to enter the resting phase (telogen) around day 20 after depilation (Fig 1*A*) (for further details see Müller-Röver *et al*, 2001). In order to induce alopecia in this mouse model, 120–150 mg per kg body weight CYP are given once intraperitoneally at day 9 after depilation (Fig 1*B*). Three to seven days later the skin is harvested for further analysis (Paus *et al*, 1994c) (Fig 1*B*).

In order to avoid terminological confusion, which is often caused by differences in how selected terms are used in hair research publications, we recently have summarized definitions of key terms employed in the context of this review (Müller-Röver *et al*, 2001). Please note that the term "proximal" here refers to those parts of the hair follicle, which are located close to the subcutaneous muscle layer, the *panniculus carnosus*, whereas "distal" refers to those parts, which are located close to the epidermis.

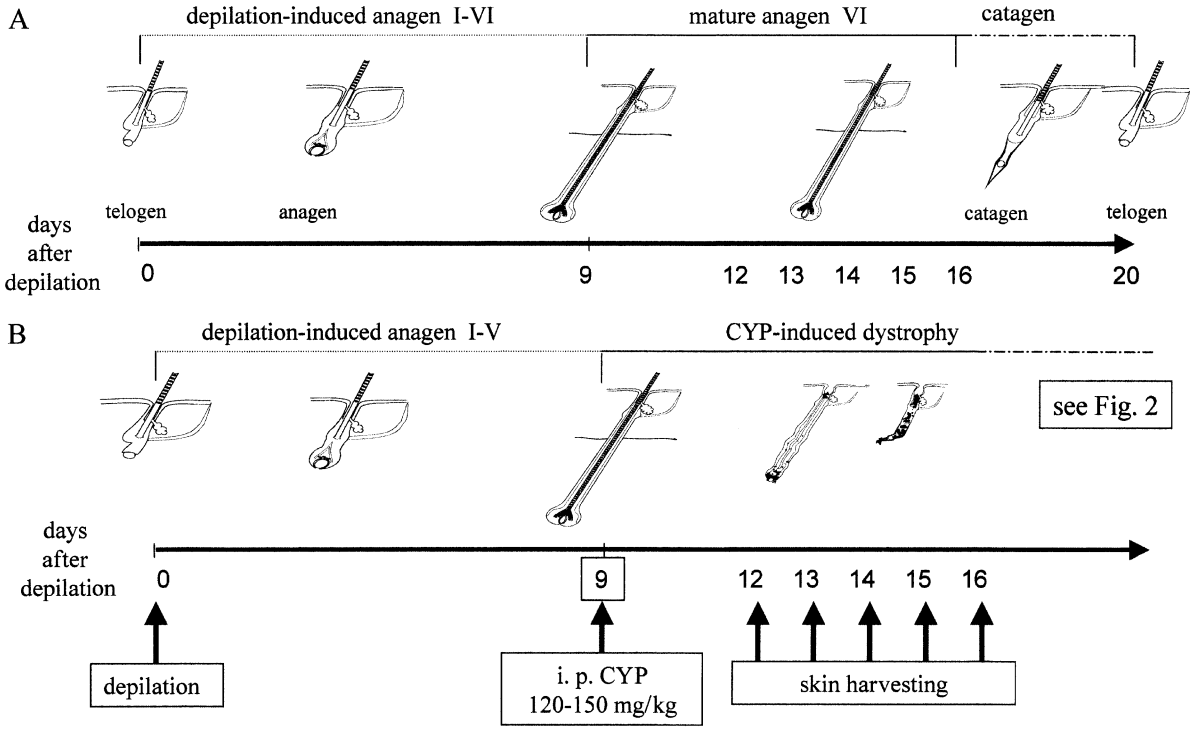

Figure 1
Experimental design of the C57BL/6 mouse models of depilation-induced haircycling and chemotherapy-induced alopecia. (*A*) The C57BL/6 mouse model of depilation-induced hair cycling. Schematic representation of key stages of depilation-induced hair follicle cycling in 7-wk-old female C57BL/6 mice. At day 0 all hair follicles are in the resting stage (telogen). Hair growth is induced by depilating the hairs with a wax/rosin mixture (for details see Chase, 1954; Paus *et al*, 1990, 1994a, b). At day 9 after depilation, all follicles are in anagen VI. Around day 16 after depilation, the first morphological signs of catagen development are detectable. At day 20 after depilation, all follicles have entered telogen again (for details see Chase, 1954; Paus *et al*, 1994a; Müller-Röver *et al*, 2001). (*B*) The C57BL/6 mouse model of cyclophosphamide-induced alopecia. Schematic representation of the experimental setup of cyclophosphamide-induced hair follicle dystrophy. At day 9 after depilation, 120–150 mg per kg body weight cyclophosphamide was injected intraperitoneally. Three to seven days after depilation the mice were killed by cervical dislocation and the skin was harvested as described (Müller-Röver *et al*, 2001).