

SCF-KIT Pathway in Human Epidermal Melanocyte Homeostasis

To the Editor:

In the August issue of the *Journal of Investigative Dermatology*, Grichnik *et al* (1998) demonstrated the responsiveness of adult human melanocytes to stem cell factor (SCF) *in vivo*. We have been interested in the role of SCF in postnatal skin since we discovered the production of SCF by human keratinocytes, and noted that the presence of the soluble form of SCF is associated with locally increased pigment production in the epidermis of patients with urticaria pigmentosa (Longley *et al*, 1993). These observations first implicated local production of SCF in postnatal melanocyte function. We agree with Grichnik *et al* (1998) that their current findings are further evidence of the fundamental role played by the SCF-KIT pathway in the regulation of melanocyte proliferation and differentiation in human postnatal skin.

We would like to take this opportunity, however, to focus discussion on the difference between the role of soluble SCF, studied by Grichnik *et al* (1995) and others (Costa *et al*, 1996; Grichnik *et al*, 1998), and the role of membrane-bound keratinocyte-associated SCF. The majority of the immunoreactive SCF found in normal human skin is bound to keratinocyte cell membranes rather than being present in a soluble form, as originally observed by us (Longley *et al*, 1993) and confirmed by others (Hamann *et al*, 1995). Membrane-bound SCF and soluble SCF have different effects on cells (Toksoz *et al*, 1992; Miyazawa *et al*, 1995), so the distinction may be important *in vivo*. In particular, based on the following observations it appears that soluble SCF is insufficient to support the normal migration and maintenance of melanocytes in the skin.

Sl/Sl^d mice, whose cells produce only soluble SCF, are white and have no melanocytes in the epidermis or hair follicles, indicating that the soluble form of the molecule is incapable of supporting melanocyte development and function (Silvers, 1979; Anderson *et al*, 1990).

In normal mice, the gene for SCF is expressed in the epidermis during development but not postnatally (Yoshida *et al*, 1996). Consequently, the number of melanocytes (and melanoblasts) present in the epidermis declines rapidly in the first few days after birth, so that essentially no melanocytes are present in the interfollicular epidermis of the adult mouse (Hirobe, 1984). The color of the adult mouse therefore is a product of melanocytes that are active in the hair follicles rather than the epidermis, which lacks melanocytes and is unpigmented. This situation differs markedly from human skin, in which epidermal keratinocytes express SCF and melanocytes are maintained postnatally. We have recently demonstrated the dominance of the membrane-associated form of SCF using transgenic mice that express SCF in the basal layer of the epidermis (Kunisada *et al*, 1998a). These studies show that the membrane-bound form of SCF is required for melanocyte survival in the epidermis, and that its expression in murine epidermis results in the population of the epidermis by melanocytes. Thus, epidermal membrane-bound SCF is both sufficient and necessary for normal melanocyte function in the epidermis. Soluble SCF, it appears, is associated with hyperfunction of melanocytes.

Also of relevance to the work of Grichnik *et al* (1996, 1998) are studies using similar strains of the transgenic mice (Kunisada *et al*,

1998b), which point to the presence of a cutaneous melanocyte 'stem cell' that, in mice, is not dependent on activation of KIT. Whether these cells do not express the KIT receptor or are merely not dependent on KIT activation for survival remains to be determined experimentally; however, it would be interesting to know whether the KIT-positive, TRP-1-negative cells described by Grichnik *et al* (1996) are dependent on KIT for their survival. One might expect to find melanocyte stem cells in the hair follicles of normal mice, because there is no indication that melanocyte stem cells are present in the interfollicular epidermis of nontransgenic mice.

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Reply

Given the current understanding of SCF expression patterns, we believe, as Drs Longley and Carter suggest, that the keratinocyte bound form of SCF will be the primary player in the control of normal human melanocyte homeostasis in the epidermis.

As Drs Longley and Carter point out, SCF has two known splice forms. Both SCF forms are initially expressed as membrane bound proteins but one can be cleaved into a "soluble" form whereas the other remains bound on the cell surface due to the absence of an exon. The region involved in KIT activation is the same for both

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molecules. Our experiments focused on the injection of the soluble form of SCF and inhibition of the KIT pathway with monoclonal antibody K44.2 (Grichnik *et al* 1998). Because both forms of SCF stimulate the KIT receptor, we believe the results of our experiments are applicable for both the soluble and the membrane bound forms of SCF. K44.2 inhibits the binding of soluble SCF to KIT (Blechman *et al*, 1995) but we have no reason to suspect that it does not also inhibit the binding of membrane bound SCF. Our findings support the importance of the SCF/KIT interaction to human melanocytes and do not exclude bound SCF.

While both forms of SCF activate KIT, we suspect that the duration of activation and potential for receptor degradation is different for each form. Keratinocytic bound SCF may lock on to the melanocyte's KIT receptor resulting in persistent KIT activation (without KIT receptor internalization and degradation), while soluble SCF may transiently activate the KIT receptor followed by internalization and degradation.

The role of KIT on the variety of melanocytic differentiation states remains for the most part unknown. It is possible that KIT expression on melanocytes is regulated through at least two mechanisms, one central (transcriptional) and one peripheral (feedback through receptor). If the central expression is low the melanocytic cells may function independently from the KIT pathway. If central expression is high, then cells become susceptible to KIT activation or inhibition. Peripheral KIT activation may also result in apparent KIT downregulation through negative feedback. Following this logic the KIT + TRP-1(-) cells (normal skin, Grichnik *et al*, 1996; nevus depigmentous, Dippel *et al*, 1995)

should be particularly susceptible to KIT inhibition; however, we have no specific data to support or refute this hypothesis.

There are still quite a number of challenges ahead. How is SCF normally regulated on human keratinocytes? How is the KIT receptor regulated on the melanocyte? Is there a functional autocrine pathway for SCF/KIT in melanocytic disease states? What are the different differentiation states of melanocytes? Which differentiation states are dependent or independent of SCF? Can these states be reversed in normal melanocytes, or in melanoma?

The myriad of different pigmentation states, nevi, and melanoma types suggests that we have a relatively complex biologic puzzle to work out. We believe the SCF/KIT pathway will factor into many of these processes at some capacity. We look forward to the next millennium and to the answers for many of these questions.

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IgG₁ and IgG₃ are the Major Immunoglobulin Subclasses Targeting Epitopes within the NC16A Domain of BP180 in Pemphigoid Gestationis

To the Editor:

Pemphigoid gestationis (PG) is a subepidermal blistering disease characterized by the linear deposition of C3 and, to a lesser extent, IgG at the cutaneous basement membrane zone (BMZ) as detected by direct immunofluorescence (IF) (Shornick, 1987). Indirect IF studies demonstrated that circulating autoantibodies of PG sera are predominantly of the IgG₁ subclass (Kelly *et al*, 1989). The autoimmune response in PG is directed against two hemidesmosomal proteins, BP180 and, less frequently, BP230 (Morrison *et al*, 1988). The pathogenic relevance of autoantibodies against BP180 in PG and bullous pemphigoid (BP) was established using a passive transfer animal model (Liu *et al*, 1993). BP180 is a transmembrane glycoprotein with a large extracellular C-terminal ectodomain containing 15 interrupted collageneous domains (Giudice *et al*, 1992; Hopkinson *et al*, 1992; Li *et al*, 1993). Immunoelectron microscopy studies have shown that the BP180 ectodomain traverses the lamina lucida and projects into the lamina densa (Bedane *et al*, 1997; Masunaga *et al*, 1997). It might interact with one or more proteins of the epidermal BMZ thus promoting the adhesion of basal keratinocytes. Recently, it was demonstrated that anti-BP180 antibodies in BP recognize four major epitopes on its membrane-proximal NC16A domain (Zillikens *et al*, 1997a), and that one of these antigenic sites (designated NC16A2 or MCW-1) is also recognized by PG sera (Giudice *et al*, 1993). The purpose

of this study was to characterize the epitopes within BP180 NC16A targeted by PG autoantibodies and to determine their subclass distribution.

Sera were obtained from 21 PG patients before treatment was initiated. All patients showed deposits of C3 and/or IgG at the BMZ by direct and/or indirect IF and demonstrated autoantibodies against BP180 NC16A by both ELISA and immunoblot analysis (Zillikens *et al*, 1997b). Twelve well-characterized BP sera and 10 normal human sera (NHS) were used as controls. Rabbit serum R58 was raised against recombinant glutathione S-transferase (GST). Reactivity of PG sera with different segments of BP180 NC16A was assayed by immunoblotting using the following recombinant GST fusion proteins: GST-NC16A1, GST-NC16A2, GST-NC16A2.5, GST-NC16A3, GST-NC16A1-3, GST-NC16A2-4, GST-NC16A2-5, and GST-NC16A1-5. Preparation of these proteins, and immunoblotting and immunoadsorption procedures were performed as described (Giudice *et al*, 1993; Zillikens *et al*, 1997a). The secondary peroxidase-conjugated antibodies were used at the following dilutions: goat antirabbit polyclonal IgG 1:5000; rabbit antihuman polyclonal IgG 1:15 000; antihuman IgM 1:5000 (all DAKO, Glostrup, Denmark); antihuman IgA 1:20 000 (Jackson ImmunoResearch Laboratories, West Grove, PA); sheep antihuman IgE 1:100; mouse antihuman IgG₁ (clone 8c/6-39) 1:1000; antihuman IgG₂ (clone HP6014) 1:500; antihuman IgG₃ (clone HP6050) 1:200; antihuman IgG₄ (clone HP6023) 1:4000 (all Binding Site, Birmingham, UK). Monoclonal antibodies were demonstrated to be specific by direct hemagglutination and hemagglutination inhibition assays (Jefferis *et al*, 1985), and by immunoblot analysis using human IgG₁, IgG₂, IgG₃, IgG₄ myeloma proteins (Sigma, St. Louis, MO). Sensitivities of secondary monoclonal

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