

Hepatocyte Growth Factor Establishes Autocrine and Paracrine Feedback Loops for the Protection of Skin Cells after UV Irradiation

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Hepatocyte growth factor (HGF) is a multifunctional cytokine, which, among various other activities, acts as a growth factor for melanocytes and has recently been implicated in the pathogenesis of malignant melanoma. In the skin, the main source for HGF is dermal fibroblasts (FB). Here, we have investigated the regulation of HGF production and secretion by cytokines derived from UV-irradiated keratinocytes (KC) and by direct UV irradiation. We demonstrate that supernatants of ultraviolet (UV)B-irradiated KC strongly induce HGF production in FB, and that this effect was mediated primarily by IL-1 α . Direct irradiation of FB with UVB had no effect on HGF expression. In contrast, irradiation with UVA₁ strongly upregulated HGF mRNA production and secretion of the functional protein. Addition of neutralizing anti-HGF antibodies after UVA₁ irradiation, as well as transfection of FB with HGF small-interfering RNA (siRNA); which completely abrogated HGF secretion led to a dramatic rise of FB apoptosis demonstrating that autocrine HGF efficiently protected FB from UVA₁-induced apoptosis. Our data suggest that upregulation of HGF plays a role in skin homeostasis after UV irradiation. However, a negative side effect of UV-induced HGF secretion by dermal FB might represent a decisive factor for induction and/or progression of melanoma.

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INTRODUCTION

Hepatocyte growth factor (HGF), a multifunctional cytokine predominantly expressed in mesenchymal cells, has various effects on cells of different origin. It was first identified as a potent mitogen for hepatocytes (Nakamura *et al.*, 1989; Montesano *et al.*, 1991), and more recently, it was shown to promote cell motility and proliferation of normal human epidermal keratinocytes (KC), melanocytes, and kidney epithelial cells (Rubin *et al.*, 1993). Furthermore, HGF is able to induce scattering of cells (Stoker and Perryman, 1985) and their invasion into extracellular matrix (Giordano *et al.*, 1993; Rong *et al.*, 1994), thereby promoting tumor metastasis

(Jeffers *et al.*, 1996; Weidner *et al.*, 1996). HGF has also been shown to have an antiapoptotic effect on different cell types. For instance, HGF acts as a survival factor after serum withdrawal for the renal epithelial cell line HKC (Liu *et al.*, 1998; Yo *et al.*, 1998) and inhibits UVB-induced apoptosis in primary KC (Mildner *et al.*, 2002). Overexpression of HGF in the skin leads to the development of pigmented skin lesions and ultimately melanoma in transgenic mice (Noonan *et al.*, 2000).

The action of HGF is mediated by its specific receptor, c-Met, a transmembrane protein encoded by the proto-oncogene, *c-met*, which has an intrinsic kinase domain (Bottaro *et al.*, 1991). After stimulation of HGF, c-Met becomes phosphorylated and initiates different intracellular signals that lead to activation of several signaling cascades such as phosphoinositide-3-kinase, mitogen-activated protein kinase, and signal transducers and activators of transcription-3 (Ponzetto *et al.*, 1994; Royal and Park, 1995; Boccaccio *et al.*, 1998).

For the skin, UV irradiation is the most important DNA-damaging insult, and represents the major risk factor for the development of epithelial skin tumors (Kraemer, 1997). Whereas mild UV-induced damage induces DNA repair, severe UV exposure leads to irreparable DNA damage resulting in KC apoptosis (Royal and Park, 1995; Schwarz *et al.*, 1995; Brash, 1996). It has been suggested that this UV-induced apoptosis contributes to the homeostasis of the epidermis and helps to prevent skin cancer by preferentially eliminating DNA-damaged KC (Brash, 1996). However, a substantial loss of KC would result in a life-threatening damage of the skin barrier function. When we recently

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Abbreviations: FB, normal human dermal fibroblasts; HGF, hepatocyte growth factor; KBM, keratinocyte basal medium; KC, normal human epidermal keratinocytes; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; PBS, phosphate-buffered saline; PI3K, phosphoinositide-3-kinase; ROS, reactive oxygen species; siRNA, small-interfering RNA; STAT, signal transducers and activators of transcription; TGF β 1, tumor growth factor beta 1; TNF α , tumor necrosis factor alpha

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investigated the regulation of UV-induced KC apoptosis, we found that HGF inhibits UV-induced apoptosis of KC. Surviving KC, however, were irreversibly arrested in the G₂/M phase of the cell cycle (Mildner *et al.*, 2002). They were therefore removed from the pool of replicating cells, thereby reducing the risk of transformation, but would still be able to contribute to the integrity of the skin barrier.

In the skin, the major source for HGF is dermal normal human fibroblasts (FB) (Stoker and Perryman, 1985). HGF production is induced in these cells after stimulation with phorbol ester (Gohda *et al.*, 1992), IL-1 α (Matsumoto *et al.*, 1992), and tumor necrosis factor- α (TNF α) (Tamura *et al.*, 1993). Since both IL-1 α and TNF α are induced after exposure of KC to UVB (Kupper *et al.*, 1987; Kock *et al.*, 1990), this could result in the triggering of HGF production by FB and the establishment of a paracrine loop between epidermal and dermal symbionts, allowing the survival of these cells after UV injury. Therefore in the present study, we investigated (1) whether soluble factors secreted by UVB-irradiated KC are able to stimulate HGF production by dermal FB, (2) whether UV irradiation leads to a direct activation of the HGF production in dermal FB, and (3) whether FB-derived HGF affects KC and/or FB survival after UV irradiation.

RESULTS

IL-1 α released by KC after UVB irradiation upregulates HGF production by dermal FB

IL-1 α and TNF α are known to be strong inducers of HGF production by FB (Matsumoto *et al.*, 1992; Tamura *et al.*, 1993). Since KCs secrete both IL-1 α and TNF α after exposure to UVB, we investigated whether supernatants from UVB-irradiated KC were able to stimulate HGF production by dermal FB. As shown in Figure S1a and b, the release of both cytokines was strongly induced by UVB irradiation (5–25 mJ/cm²). When the culture supernatants derived from irradiated KC 24 hours after irradiation were transferred onto non-irradiated FB, HGF production was strongly upregulated (Figure 1a). Upregulation of HGF mRNA was completely prevented when the KC supernatant was treated with a neutralizing anti-IL1 α antibody (Figure 1b), whereas a neutralizing anti-TNF α antibody had no effect (data not shown).

FB-derived HGF inhibits UVB-induced KC apoptosis

To test whether FB-derived HGF, like recombinant HGF, is able to prevent UVB-mediated apoptosis of KC, we transferred culture supernatants of FB 48 hours after stimulation with supernatants derived from UVB-irradiated KC to cultures of freshly UVB-irradiated KC. Both recombinant HGF and conditioned cell-culture supernatants from FB treated with KC supernatants irradiated either with 20 or 25 J/cm² UVB comparably inhibited UVB-induced apoptosis of KC, whereas medium alone had no protective effect (Figure 2a). Treatment of KC with HGF alone showed no morphological differences compared to non-irradiated KC (Figure 2a). Cell-culture medium of untreated FB or treated with supernatants of non-irradiated KC showed no antiapoptotic activity (data not shown). These data were confirmed by histone ELISA, which also showed a strong inhibition of UVB-induced KC apoptosis

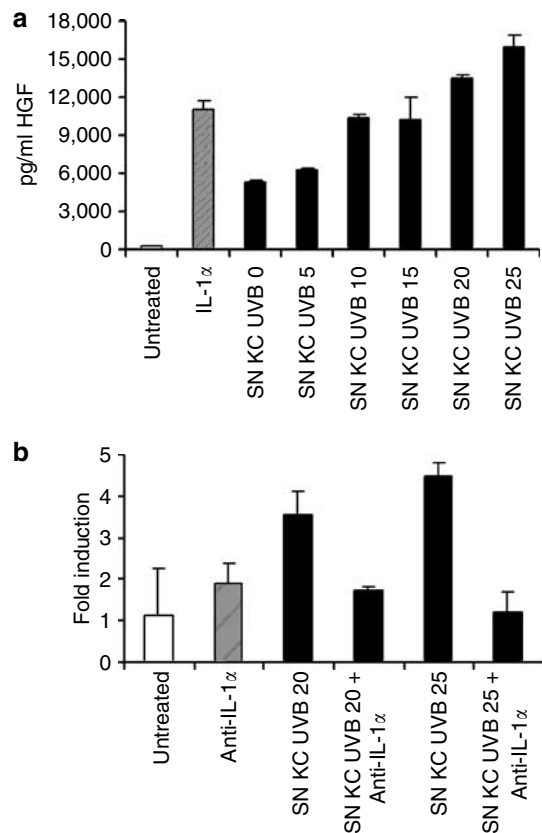


Figure 1. IL-1 α produced by UVB-irradiated KC induces HGF production in dermal fibroblasts. (a) FBs were treated with cell-culture supernatants from KC exposed to 0–25 mJ/cm² UVB or with IL-1 α . Forty-eight hours after treatment, the amount of secreted HGF in the cell-culture supernatant was analyzed by HGF ELISA. (b) FB were exposed to cell-culture supernatants from KC irradiated with 20 or 25 mJ/cm² UVB either in the absence or presence of a blocking anti-IL-1 α antibody. Eight hours after stimulation, RNA from FB was prepared and real-time PCR for HGF mRNA was performed. One representative experiment of three is shown. Error bars represent 1 SD calculated from three replicates for each set of values.

by conditioned FB cell-culture supernatants (Figure 2b), whereas supernatants from unstimulated FB had no antiapoptotic activity (data not shown). The antiapoptotic effect of the supernatants was due to FB-derived HGF, since it could be almost entirely neutralized by the addition of an anti-HGF antibody (Figure 2c).

HGF is strongly induced in FB after UVA₁ irradiation and protects them against UVA₁-induced apoptosis in an autocrine manner

To study whether UV irradiation by itself could induce HGF secretion by FB, we irradiated these cells with UVB and UVA₁. As shown in Figure 3, UVB at 5–20 mJ/cm² did not upregulate HGF mRNA expression (Figure 3a) and protein production (Figure 3c) in FB. Although absolute values varied between individual experiments, the relative induction by the different stimuli was consistently comparable. Moreover, UVB irradiation inhibited the increase of the baseline HGF mRNA production, which increased during 24 hours in non-

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