Epidermal Elafin Expression Is an Indicator of Poor Prognosis in Cutaneous Graft-versus-Host Disease

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Graft-versus-host disease (GVHD) remains a common and potentially life-threatening complication of allogeneic hematopoietic stem cell transplantation. In the skin, GVHD can present in an acute (aGVHD), chronic lichenoid (clGVHD), or chronic sclerotic form (csGVHD). Measuring peripheral blood levels of the keratinocyte-derived protease inhibitor elafin has recently emerged as a promising tool for the diagnosis of cutaneous aGVHD. We evaluated whether the analysis of elafin expression in skin would allow distinguishing aGVHD from drug hypersensitivity rashes (DHR) and whether cutaneous elafin expression would correlate with disease severity or altered prognosis of aGVHD and clGVHD/csGVHD. Skin biopsies from aGVHD ($n=22$), clGVHD ($n=15$), csGVHD ($n=7$), and DHR ($n=10$) patients were collected and epidermal elafin expression and its association with diverse clinical/histological parameters were analyzed. Acute GVHD and DHR displayed varying degrees of elafin expression. No elafin was detectable in csGVHD, whereas the molecule was increased in clGVHD as compared with aGVHD. Elafin-high aGVHD/clGVHD lesions presented with epidermal thickening and were associated with poor prognosis—i.e., decreased overall survival in aGVHD and corticosteroid resistance in clGVHD. Although cutaneous elafin does not seem to discriminate aGVHD from DHR lesions, our study strongly suggests an association between cutaneous elafin expression and poor prognosis for patients with cutaneous GVHD.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HCT) is a potentially curative therapy for numerous hematological disorders. The broader application of HCT is limited by its most frequent and potentially life-threatening complication, namely graft-versus-host disease (GVHD; Ferrara *et al.*, 2009). GVHD can occur in an acute or a chronic form (aGVHD and cGVHD), in both of which the skin is the most frequent and usually the first affected organ (Higman and Vogelsang, 2004; Ferrara *et al.*, 2009). Despite major progresses in HCT practice, available treatment options for GVHD patients are limited and corticosteroids remain the first-line therapy for both aGVHD and cGVHD (Wolff *et al.*, 2010, 2013). Early diagnosis of skin involvement in GVHD is crucial for an appropriate therapy in an attempt to prevent disease progression.

Skin aGVHD usually presents as a maculopapular or morbilliform rash (Wagner and Murphy, 2005; Hausermann *et al.*, 2008). Histopathological features of aGVHD include satellite cell necrosis (dyskeratotic keratinocytes surrounded by lymphocytes) and the vacuolization of basal epidermal layers. Chronic cutaneous GVHD shares major characteristics with lichen planus during the early stages of disease progression (chronic lichenoid GVHD, clGVHD). Clinically, this is evidenced by the presence of lichenoid papules. Histopathologically, clGVHD shows a wedge-shaped hypergranulosis and basal cell liquefaction of the epidermis, as well as a prominent band-like lymphocytic infiltrate in the dermis. In a more advanced stage, cGVHD tends to exhibit signs of sclerosis (chronic sclerotic GVHD, csGVHD).

It is challenging both clinically and histopathologically to differentiate between aGVHD and its main differential diagnosis, namely drug hypersensitivity rashes (DHR). This distinction has critical therapeutic consequences—i.e., either the

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Abbreviations: aGVHD, acute graft-versus-host disease; clGVHD, chronic lichenoid graft-versus-host disease; csGVHD, chronic sclerotic graft-versus-host disease; DHR, drug hypersensitivity rashes; HCT, allogeneic hematopoietic stem cell transplantation; HE, hematoxylin and eosin; IH, immunohistochemistry; TNFα, tumor necrosis factor alpha; TGFβ, transforming growth factor beta

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initiation of a systemic corticosteroid regimen to control GVHD or the discontinuation of the drug(s) causing DHR. Numerous approaches have aimed at identifying parameters to diagnose aGVHD more accurately and to distinguish it from DHR (Byun *et al.*, 2011). Elafin has (in a retrospective proteomic approach) been identified as an accurate plasmatic biomarker of cutaneous aGVHD that correlates with disease severity and even has a prognostic value (Paczesny *et al.*, 2010; Levine *et al.*, 2012). The investigators also showed that elafin was overexpressed in seven out of ten aGVHD skin specimens but was absent in a similar number of DHR biopsies (Paczesny *et al.*, 2010).

Elafin is a serine protease inhibitor that is mostly produced by epithelial cells. In the skin, keratinocytes are the main source of this molecule. Although elafin is not detectable in normal skin, it is secreted abundantly in psoriasis and other inflammatory (Tanaka *et al.*, 2000; Kamsteeg *et al.*, 2010), as well as neoplastic (Alkemade *et al.*, 1993), skin disorders. Elafin acts in various ways on the cutaneous immune homeostasis by not only exerting antiprotease effects but also immunomodulatory and antiproliferative ones (Williams *et al.*, 2006; Verrier *et al.*, 2012). Hence, keratinocyte-derived elafin seems to favor the development of a T helper type1 response (Roghanian *et al.*, 2006), but it can also enhance the resolution of inflammation by facilitating phagocytosis of apoptotic leukocytes (Williams *et al.*, 2006).

In this study, we sought to analyze cutaneous elafin expression in the acute and chronic forms of GVHD and to explore whether cutaneous elafin was associated with distinct histopathological or clinical features related to the severity or outcome of GVHD. In addition, we wanted to evaluate the potential of cutaneous elafin as a marker to distinguish aGVHD from DHR.

RESULTS

Varying degrees of elafin expression in aGVHD and DHR

To explore the potential of cutaneous elafin as a marker to distinguish aGVHD from DHR, we quantified cutaneous elafin in these two diseases at both the mRNA and protein levels. We performed a quantitative real-time polymerase chain reaction (reverse transcriptase-PCR) with RNA extracted from whole-skin biopsies of 22 aGVHD patients, as well as 10 DHR patients. This analysis of elafin mRNA levels (normalized to healthy control skin) did not reveal any difference between aGVHD ($n=22$) and DHR ($n=10$; Figure 1a), and we could confirm our data in an independent cohort of aGVHD patients (Supplementary Figure S1 online).

In accordance with this finding, immunohistochemistry (IH) staining for elafin on paraffin sections of the same patients revealed various elafin expression patterns (Figure 1b and e). In some sections, elafin was restricted to the stratum granulosum, whereas in others it extended to the stratum spinosum (Figure 1e). In contrast, healthy control skin samples ($n=7$) were completely negative, and lesional psoriasis skin (stained as a positive control, $n=5$) was highly positive for elafin (Figure 1c). The percentage of elafin-positive keratinocytes as quantified using the HistoFAXS software (TissueGnostics GmbH, Vienna, Austria) was not significantly higher in aGVHD as compared with DHR (Figure 1d).

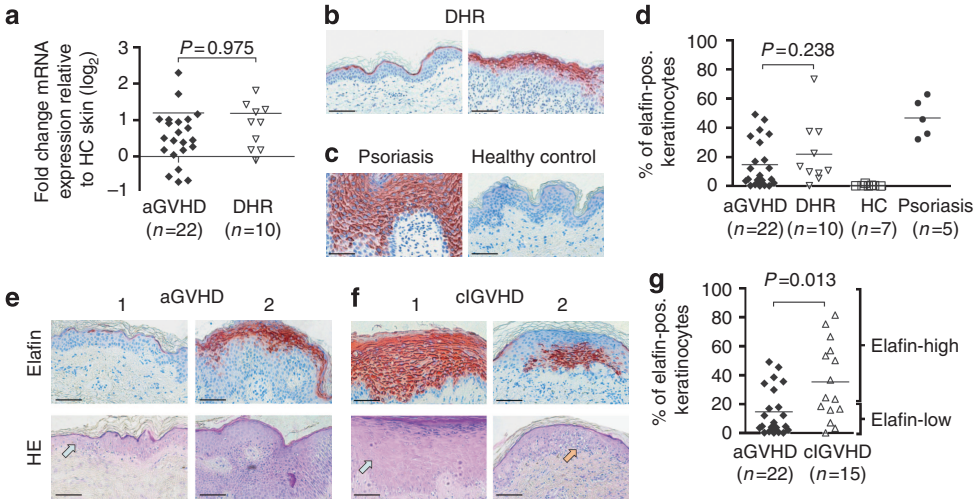

Figure 1. Various elafin expression patterns in cutaneous graft-versus-host disease (GVHD). (**a**) mRNA expression of elafin in acute graft-versus-host disease (aGVHD) and drug hypersensitivity reaction (DHR), normalized to healthy control (HC) skin. Quantitative real-time reverse transcriptase-PCR (RT-PCR) was performed after TRizol lysis of skin biopsies. (**b**, **c**) Representative pictures (scale bar = 100 μm in all pictures) of immunohistochemistry (IH) elafin staining in DHR, HC, and psoriasis. Images were taken using a PixeLINK PL-B623CF color digital camera (Zeiss, Oberkochen, Germany). (**d**) The percentage of elafin-positive keratinocytes in aGVHD as compared with DHR measured using HistoQuest imaging analysis software. Expression in HC and lesional psoriatic skin is shown as a negative control and a positive control, respectively. (**e**, **f**) Pictures of elafin staining in aGVHD and chronic lichenoid graft-versus-host disease (clGVHD) skin (two different patients each) with corresponding hematoxylin and eosin (HE) sections shown below (scale bar = 100 μm in all pictures). Blue arrows indicate correlation of epidermal thickness with increased elafin expression. The orange arrow points to the prominent inflammatory infiltrate in an elafin-high skin area. (**g**) Percentage of elafin-positive keratinocytes in aGVHD vs. clGVHD. Cutoff (at 20%) between the elafin-high and the elafin-low groups.