Histone Deacetylase 2 Is Upregulated in Normal and Keloid Scars

Journal of Investigative Dermatology advance online publication, 29 December 2011; doi:10.1038/jid.2011.432

TO THE EDITOR

Repair following cutaneous injury is essential to return function, form, and integrity to the tissue. Scar formation is the inevitable and currently unpreventable consequence of tissue damage, and misregulation can lead to the development of pathological scarring as in hypertrophic and keloid scars. In developed countries alone, approximately 100 million people each year will be left with a scar following surgery; moreover, there are thought to be 11 million people with keloid scars (Sund, 2000). This represents a significant burden to both health-care providers and individuals.

Currently, the pathogenesis of keloid scars is not fully understood, but there are a number of clues as to the etiology. There is an obvious genetic component, and they have also been linked to hormonal influences, wound infection, and skin tension (Wolfram *et al.*, 2009). Given the burden presented and our current poor ability to reduce or prevent normal and pathological scarring, clearly an improved understanding of the molecular mechanisms underlying scar formation is essential, and research into novel treatment strategies is justified.

Histone deacetylases (HDACs) and counteracting histone acetyltransferases (HATs) are epigenetic modifying enzymes that are accepted to, respectively, remove and add acetyl groups to histones, and in this manner influence gene expression (acetylation is generally an activating, transcription-promoting modification; Kuo and Allis, 1998). The objective of this work was to characterize the expression profiles of specific HDACs in normal and keloid scars; this has not been previously investigated, and there is a lack of consensus about the role of histone (de)acetylation in various fibrotic conditions. For example, HDAC inhibitors used *in vitro* suppressed myofibroblast differentiation (Glenisson *et al.*, 2007; Mannaerts *et al.*, 2010) and decreased collagen production in keloid fibroblasts (Diao *et al.*, 2011); in animal models, HDAC inhibition decreased heart, kidney, and liver fibrosis (Pang *et al.*, 2009; Iyer *et al.*, 2010; Marumo *et al.*, 2010). Conversely, HATs were overexpressed in fibrotic lesions of scleroderma patients (Bhattacharyya *et al.*, 2005), and HAT inhibition was found to be anti-fibrotic (Li *et al.*, 2008).

To investigate HDAC expression in human skin scars, three types of skin tissue were assessed by immunohistochemistry (IHC): normal human skin, normal scar tissue from patients undergoing melanoma re-excision approximately 2–3 weeks after the original wound, and keloid scar tissue from revision procedures (age of scar >6 months). Tissue samples were batch analyzed for HDAC1, HDAC2, HDAC4, and HDAC7. Scar-associated fibroblasts in both normal and keloid scars showed a significant and striking upregulation of HDAC2, but not HDAC1, 4, or 7 (Figure 1). Within the epidermis, HDAC expression was unchanged in scar versus non-scar regions (data not shown).

Our observation that HDAC2 was upregulated in scar tissue was substantiated using a mouse model of wound repair. Specifically, 4-mm excisional wounds were made to the shaved dorsal skin of anesthetized adult male mice (CD-1; 6–8 weeks; protocol approved by an institutional ethics committee and the UK Home Office). Wound tissue was harvested after 3, 7, or 14 days and analyzed for HDAC2 by IHC. On Day 3, HDAC2-positive cells were found at the wound margins, whereas on Days 7 and 14 highly expressing cells were abundant in the wound bed (Figure 2a). This approach demonstrated that HDAC2 is upregulated in normal scar tissue in the mouse as it is in human.

To begin to understand how HDAC2 expression may be regulated in this setting, primary cultures of normal human dermal fibroblasts (nHDFs, $<$ passage 15) were either (1) treated with transforming growth factor (TGF)β1 (0, 0.1, 0.5, 1 ng ml^{-1}) or (2) cultured at varying densities, and the effects on HDAC2 expression were observed by western blot analysis. TGFβ1 was able to trigger a concentration-dependent upregulation of HDAC2 (Figure 2b). Intriguingly, this effect was not observed in all patients: only two of the four primary cell populations tested responded in this way. Future work will investigate the determinants of this variable response and its influence on the efficacy of different targeted therapeutics. The same two nHDF isolations showed that HDAC2 expression correlates positively with cell density (Figure 2b), which would be high in a developing scar *in vivo*. Experiments using murine Swiss 3T3 fibroblasts ($<$ passage 12) confirmed that TGFβ1 can increase HDAC2 expression, and, interestingly, also revealed that HDAC1 and HDAC7 were TGFβ1-responsive in these cells (Figure 2c). This seemingly contradictory finding that only HDAC2 was upregulated in *in vivo* scars, whereas TGFβ1 stimulation of cultured fibroblasts increased HDAC1 and 7, as well as 2, may indicate that the timing and duration of TGFβ1

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HAT, histone acetyltransferase; HDAC, histone deacetylase; IHC, immunohistochemistry; TGFβ, transforming growth factor-β

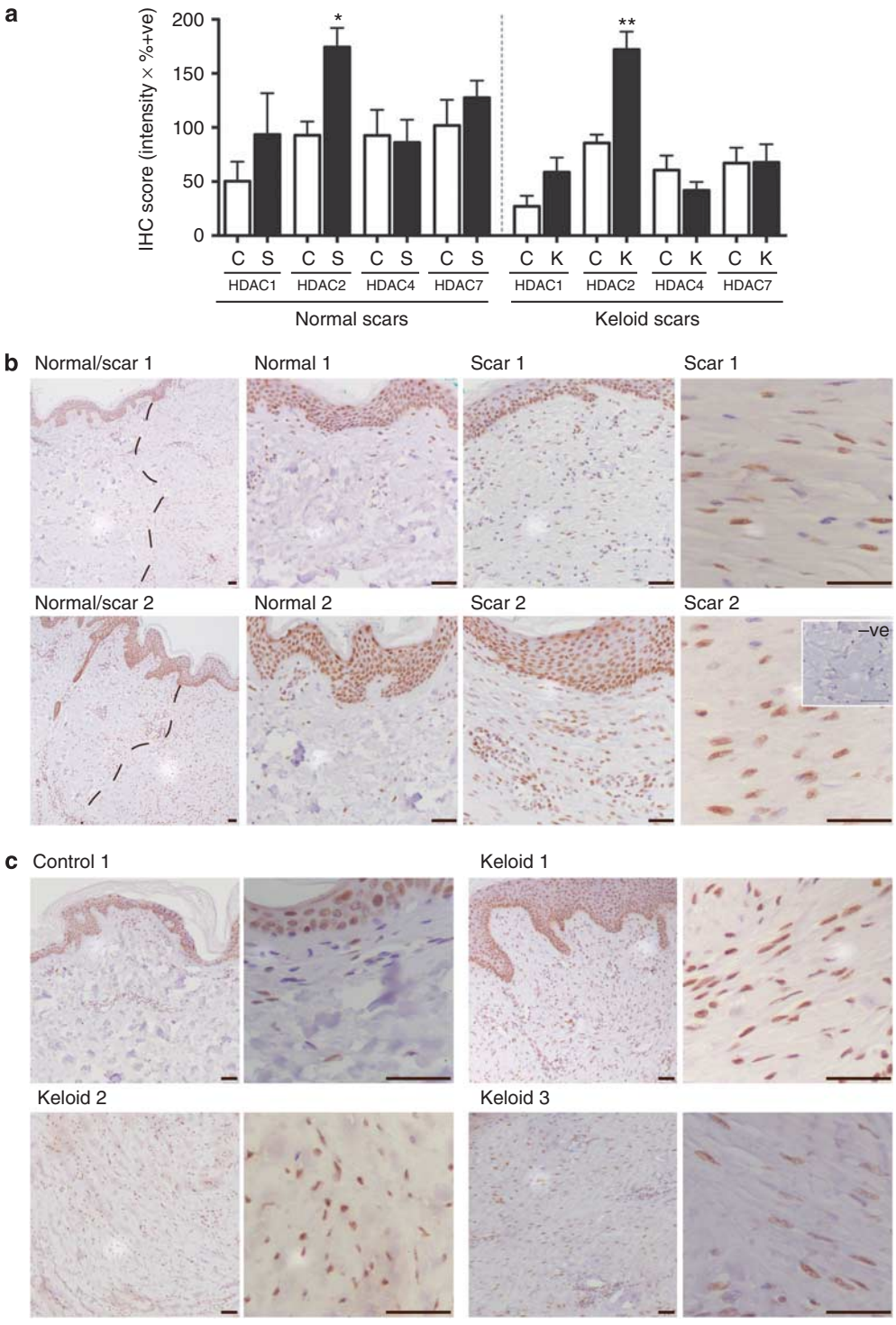

Figure 1. Immunohistochemistry (IHC) reveals histone deacetylase (HDAC)2 upregulation in human scar tissue. (**a**) Formalin-fixed, paraffin-embedded human scar samples were analyzed by IHC for expression of HDAC1, 2, 4, and 7. At least three independent reviewers scored the staining intensity and the percentage of positive dermal fibroblast cells in normal skin and scar tissue. The product of these two numbers (mean of 3/4 scorers) gave the IHC score, which was statistically analyzed and is graphically represented. Results were analyzed by paired (normal scars) or unpaired *t*-tests (keloids); *, $P<0.005$ ($n=10$); **, $P<0.0001$ ($n=11$). (**b**, **c**) Representative patient samples showing HDAC2 upregulation in (**b**) normal scar tissue, and (**c**) keloid scars are shown. Brown staining indicates HDAC2 positivity, omission of the primary antibody served as a negative control. Bars = 50 μm.

exposure are important factors in the regulation of specific family members; alternatively, there may be confounding negative influences on the expression of specific HDACs in the heterogeneous *in vivo* setting.

Our *in vivo* studies on mouse and human skin wounds revealed that HDAC2 is significantly overexpressed

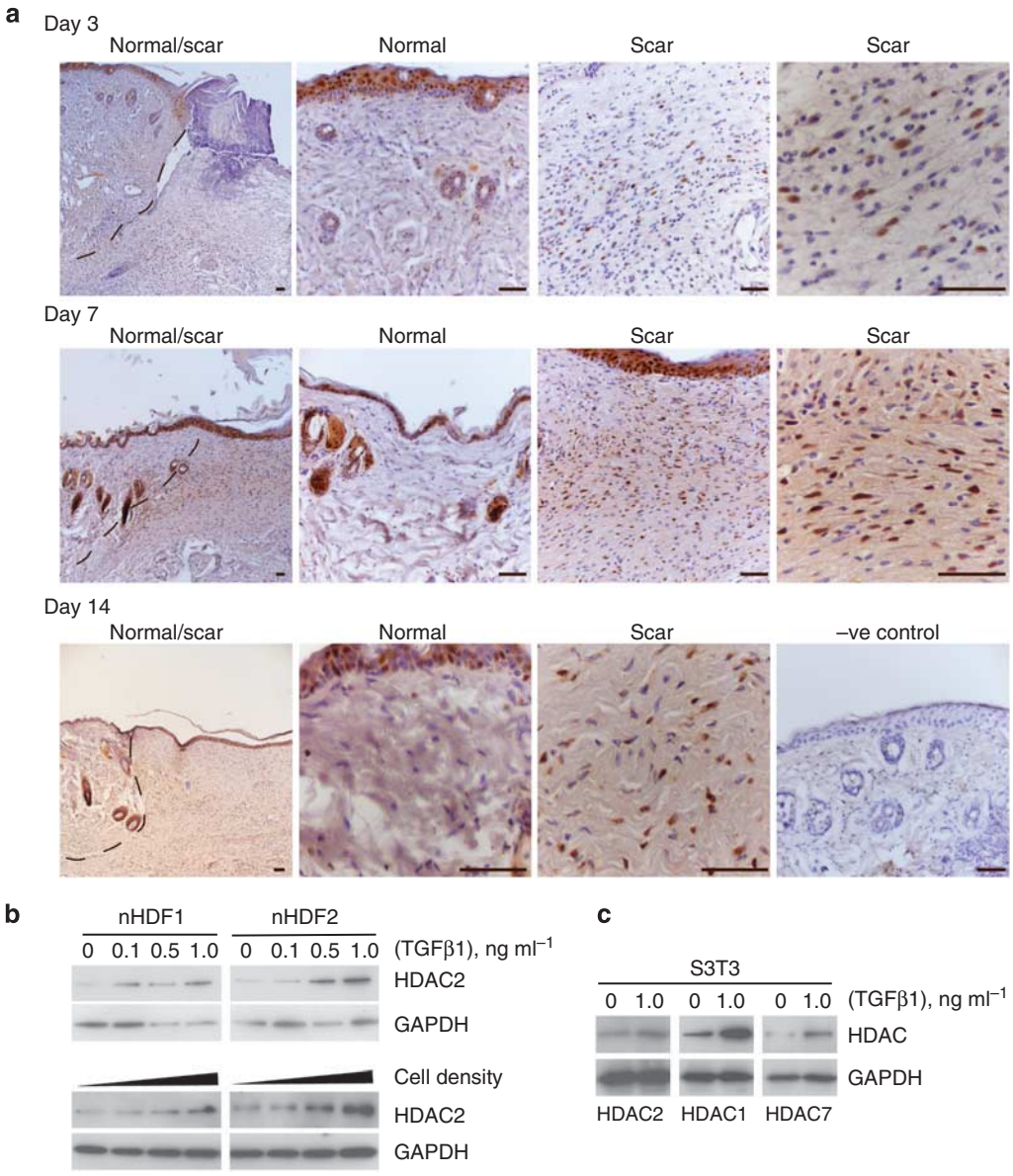

Figure 2. Transforming growth factor (TGF)β1 and cell density may regulate histone deacetylase (HDAC)2 expression in scar tissue. (**a**) Immunohistochemistry of scars resulting from a 4-mm punch biopsy wound made to the dorsal skin of adult male mice showed elevated HDAC2 expression (brown) in the scar-associated fibroblasts throughout the time course of scar formation. Omission of the primary antibody served as a negative control. Bars = 50 μm. (**b**) Western blot analysis of normal human dermal fibroblasts (nHDF, < passage 15) treated with the indicated concentrations of TGFβ1 for 24 h, or cultured at varying densities (approximately 25, 50, 75, and 100% confluence) for 96 h, revealed a "concentration"-dependent increase in HDAC2 expression. (**c**) TGFβ1 (1 ng ml^{-1}) stimulation of Swiss 3T3 (S3T3) cells also resulted in upregulation of HDAC2, as well as an increase in HDAC1 and 7. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a loading control.

in both normal and keloid scar tissue. The failure of HDAC2 to distinguish between normal and keloid scars leads us to speculate that, in keloids, expression may fail to return to normal levels at the completion of the healing process, and thus may contribute to persistent growth and/or alternative differentiation of these cells.

Our ongoing hypothesis is that pharmacological inhibition of HDACs will decrease skin fibrosis. Early, but promising, findings in various animal models of fibrosis certainly indicate that this may be the case in other organ systems (Pang *et al.*, 2009; Iyer *et al.*, 2010; Marumo *et al.*, 2010). As inflammation tends to exacerbate scarring, it will be interesting to determine whether these results are owing to HDAC inhibitors acting directly on the fibroblasts, or acting indirectly, as anti-inflammatories (Han and Lee, 2009). Regardless of the mode of action, if correct, the pathway from bench to bedside for HDAC inhibitors in the treatment of skin scars is anticipated to be relatively smooth, as there are 13 HDAC inhibitors already in use clinically (Paris *et al.*, 2008).

Currently, there are no successful treatments that prevent or eliminate scar tissue; however, counteracting TGFβ1,

for example, using recombinant TGFβ3 (Avotermin, Renovo, UK), has shown potential success as an anti-scarring treatment (So *et al.*, 2011). Our finding that TGFβ1 treatment increased HDAC expression suggests that HDAC inhibitors equally have the potential to be anti-fibrotic, depending on the extent to which TGFβ1 relies on HDAC2 to exert its effects. People with scars face many physical, psychological, esthetic, and social consequences that may be associated with substantial emotional and financial cost (Brown *et al.*, 2008). This research implicates HDACs in skin scarring, and suggests they may be novel therapeutic targets for the prevention of normal and pathological scarring.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the University of London Central Research Fund and St George's, University of London.

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