Identification of a Potential Molecular Diagnostic Biomarker in Keloid Disease: Syndecan-1 (CD138) Is Overexpressed in Keloid Scar Tissue

TO THE EDITOR

The diagnosis between different forms of raised dermal scarring such as hypertrophic (HTS) and keloid scars (KS) can be difficult in the absence of a specific biomarker (Atiyeh, 2007; Lee et al., 2004). Importantly, the differentiation can be critical before initiation of treatment, as misdiagnosis followed by inappropriate therapy may result in worsening of the outcome by increasing rate of recurrence in keloids (Viera et al., 2012). Therefore, the aim here was to identify a molecular diagnostic biomarker capable of distinguishing KS from other forms of
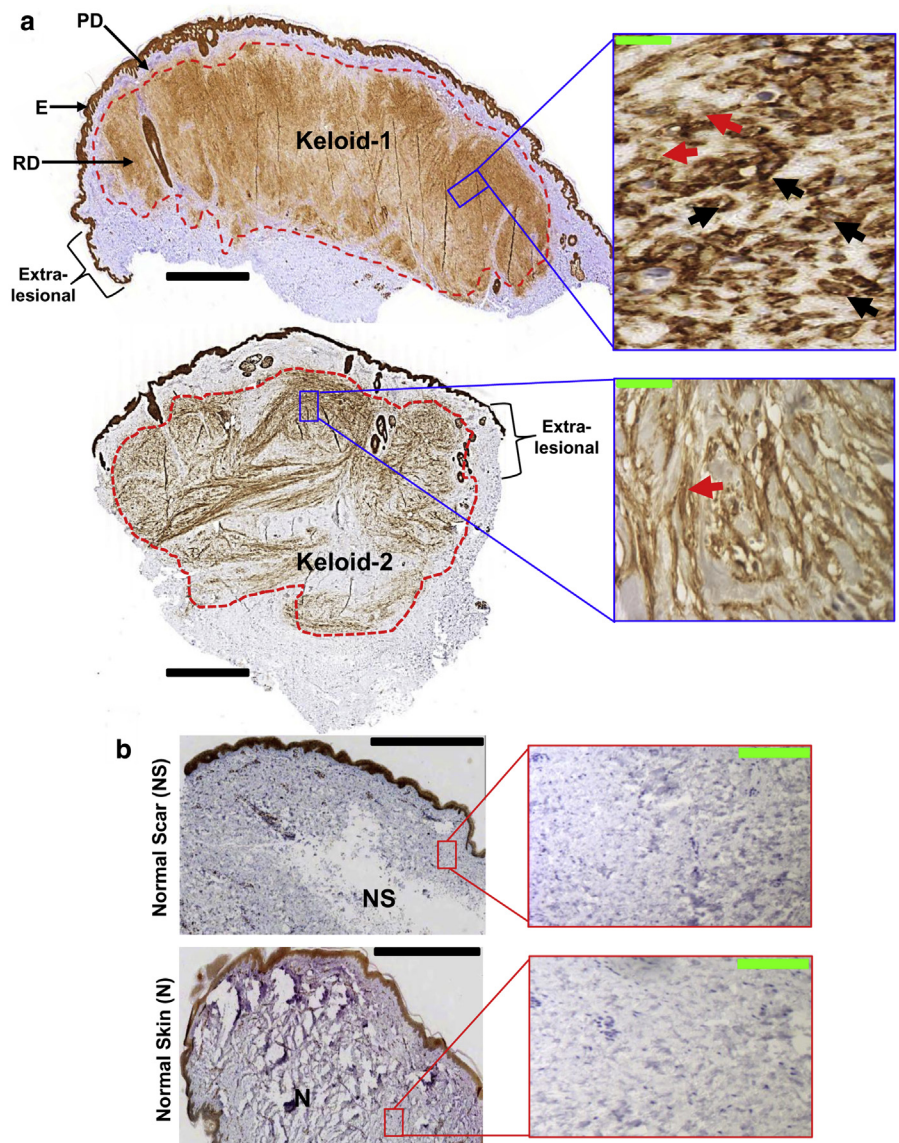
Figure 1. Representative immunohistochemical micrographs of syndecan-1 (CD138) overexpression in keloid compared with normal scar and normal skin tissue. There is overexpression of syndecan-1 immunoreactivity within keloid reticular dermis, sparing papillary dermis; scale bar (black) = 1 mm and scale bar (green) = 50 μm (**a**), relative to NS and normal skin; scale bar (black) = 500 μm and scale bar (green) = 100 μm (**b**). Expression is observed on cells (black arrows) and to some extent in extracellular matrix (red arrows). Paraffin-embedded tissues for 65 keloid samples, 11 NS, and 11 normal skins were sectioned at 5 μm. Then, they were dewaxed, dehydrated, and labeled with a syndecan-1 antibody and detected using chromogen. Slides were scanned using ScanScope (Aperio Technology, Oxford, UK). Micrographs were captured at 8 magnifications, and inserts were captured at 200 and 400 magnifications. E, epidermis; KS, keloid scar; N, normal skin; NS, normal Scar; PD, papillary dermis; RD, reticular dermis.

dermal scarring specially HTS and also dermatofibrosarcoma protuberans (DFSPs)—an uncommon progressive, reticular dermal neoplasm with high recurrence. Often, keloid and/or DFSP are misdiagnosed (Kimura et al., 2014, Nguyen et al., 2002, Nicholas and Stodell, 2014, Suarez et al., 2015). We have demonstrated immune upregulation and the presence of lymphoid aggregates while investigating the presence of plasma cells in 63 KS tissue samples (Bagabir et al., 2012) using immunohistochemistry, and serendipity that led to the identification of abundant expression of CD138 in KS. Localized abundant expression of CD138 within the keloid lesion had drawn our attention to further understand the link between CD138 and keloid.

CD138 (syndecan-1) is a cell surface proteoglycan, which is shed by the expressing cells into the extracellular matrix (Szatmári et al., 2015). Syndecan-1 is present in neonatal scars, while its lack of expression in scar-less fetal wound healing (Gallo et al., 1996) suggests a potential functional role for syndecan-1 in fibrosis in scar formation. Interestingly, syndecan-1 binds to the collagens and fibronectin deposited during tissue repair, as a consequence of which the tissue reverts to a quiescent state (Szatmári et al., 2015). It modulates cell proliferation, migration, and tumor progression in cancer cells (Szatmári et al., 2015), and regulates keratinocyte proliferation and the proteolytic balance in healing wounds (Stepp et al., 2015).

To follow up the overexpression of syndecan-1 in keloid and to probe the hypothesis that it may be a unique, previously unreported, biomarker, its expression in KS was compared with HTS, DFSP tumor, normal scar (NS), and normal skin. For all the tissue samples used in this study, ethical approval was obtained from NHS Ethical Committee, Manchester, UK. All cases recruited gave full informed verbal and written consent to take part in the study (ethical reference number 11/NW/0638). The in situ staining of keloid (n = 65), NS (n = 11), DFSP (n = 10), HTS (n = 3) (see Supplementary Material and Methods online for details), and normal skins (n = 11) showed a clear overexpression of syndecan-1 in keloid when compared to HTS, DFSP, NS, and normal skin. Keloid cross-sections revealed an intensive immune reactivity of syndecan-1 within keloid reticular dermis (Figure 1a and Supplementary Figure S1 online), whereas perilesional skin lacked its expression. Surprisingly, in situ syndecan-1 expression was mild to absent in HTS and NS, respectively, and completely absent in healthy skin and in DFSP tumors when compared with keloid (Figures 1b and 2a and Supplementary Figures S1–S5 online). However, the expression pattern of syndecan-1 was variable in between the keloid samples from different patients and/or different anatomical sites (Supplementary Figure S1 and Table S1 online). Minimal to no expression of syndecan-1 was detected in HTS
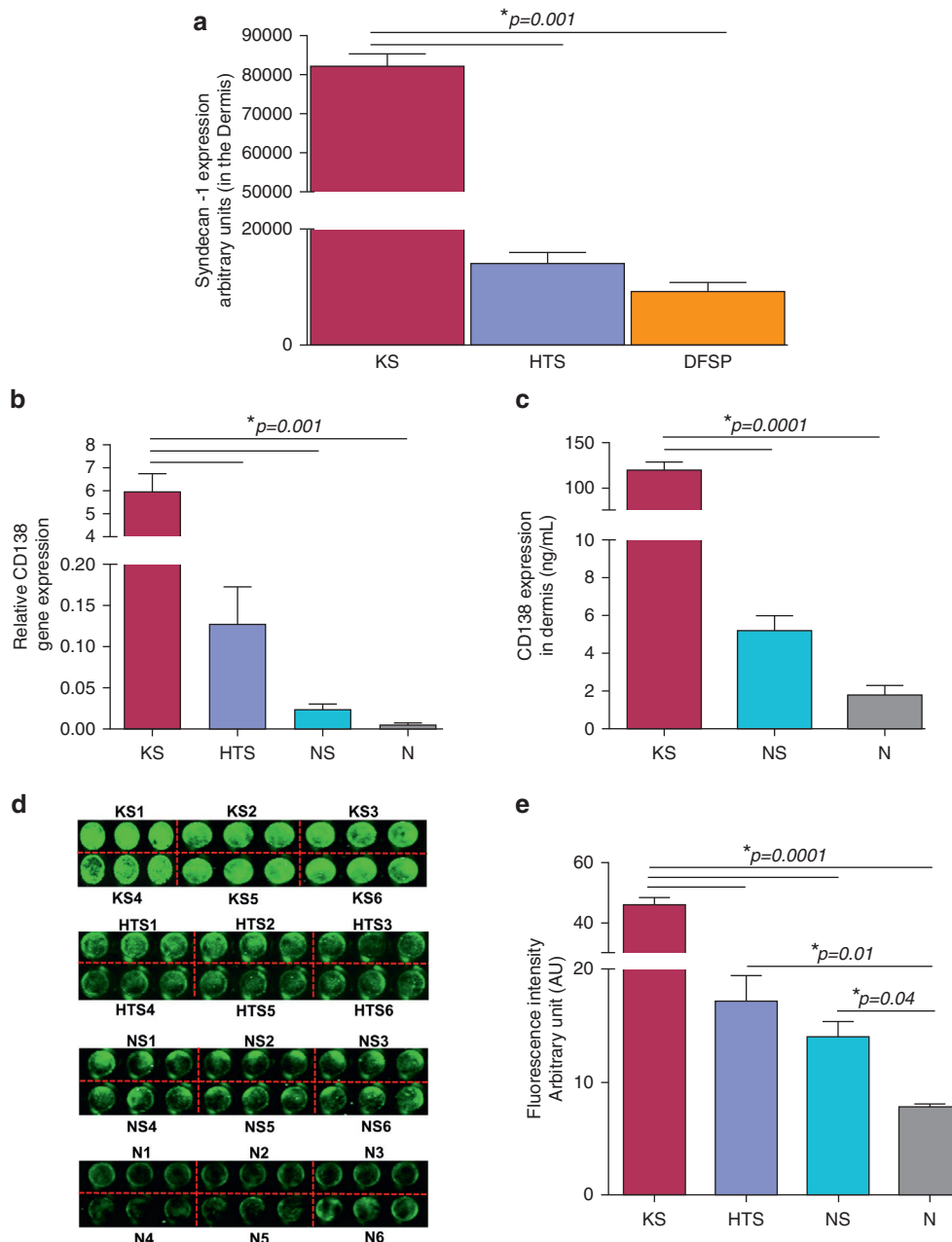
Figure 2. Significant overexpression of syndecan-1 (CD138) at transcriptional and translational level in keloid tumor compared with normal scar and normal skin. (**a**) Bar graphs represent the immunomorphometric quantitative analysis of CD138 expression in keloid (KS, n = 9), hypertrophic scars (HTS, n = 3), and in DFSP (n = 10) tumor tissue samples. A significant increase in syndecan-1 protein expression (*$P = 0.001$) was detected in keloid as compared with HTS and DFSP tumor tissue. (**b**) Overexpression of mRNA in keloid intralesional tissue compared with hypertrophic scar (HTS), normal scar (NS), and normal skin (N) dermal tissue. Fold change of syndecan-1 mRNA in the KS-defined lesional site relative to HTS, NS, and normal skin. Primers for syndecan-1 were designed (Supplementary Table S5 online). mRNA was extracted from full-thickness tissue derived from defined KS site intralesion (n = 9), HTS (n = 4), NS dermis (n = 8), and normal skin dermis (n = 7). Two micrograms of mRNA was reverse transcribed and the quantitative real-time RT-PCR reaction was carried out. The cycle threshold (Ct) values were normalized to the geometric mean of two housekeeping genes (SDHA: succinate dehydrogenase complex subunit A and RPL32: 60S ribosomal protein L32) and analyzed by fold change ($2^{-\Delta\Delta CT}$) relative to NS and normal skin. Error bars represent mean ± SEM. (**c**) Ex vivo quantitative measurement of syndecan-1 in keloid, NS, and normal skin dermal lysates. Syndecan-1 protein was extracted from keloid (n = 8) intralesional site (core of the keloid tissue) and dermis of NS (n = 6) and normal skin (n = 6), using modified radioimmunoprecipitation assay buffer. ELISA was performed in triplicate as described in Supplementary Material and Methods. Results are presented as mean ± SEM. (**d**) Syndecan-1 expression profile in keloid (core region of keloid), HTS, NS, and normal skin primary fibroblasts isolated from the dermis. Serum-starved keloid fibroblasts were seeded in 96-well plates (1.0×10^4 cells/well) and grown up to 24 hours. The cells were then fixed with 4% formaldehyde/phosphate-buffered saline. After blocking, syndecan-1 was detected by incubation with anti-CD138, followed by incubation with an IRDye 800-labeled secondary antibody. The representative output infrared image of a 96-well plate shows the syndecan-1 expression pattern across different fibroblast cell lines. (**e**) Bar graphs represent the quantification of mean syndecan-1 protein expression in KS, HTS, NS, and normal skin primary fibroblast cell lines. Results are presented as mean ± SEM of triplicates (n = 6). HTS, hypertrophic scars; KS, keloid scar; N, normal skin; NS, normal scar; SEM, standard error of the mean.

and NS dermis, but consistent higher expression was seen in keratinocytes and endothelial cells in all the tissue samples including DFSP (Supplementary Figures S2—S5). Interestingly, syndecan-1 immunoreactivity was localized within the keloid tumor mass, keloid stroma, and epidermis, sparing papillary dermis. Its expression was both cellular and in the extracellular matrix. However, the expression of syndecan-1 was absent in the dermis in normal skin adjacent to keloid tissue (extralesional) (Figure 1a and Supplementary Figure S1).

To further corroborate the above findings and to compare CD138 expression between KS and HTS, we assessed its mRNA and protein expression in KS (n = 13), HTS (n = 10), NS tissue (n = 9), and healthy skin (n = 8) by quantitative RT-PCR, ELISA, and in-cell western blotting (see Supplementary Material and Methods for details, and Supplementary Tables S1—S4 online for patient demographic data). The results corresponded with a significant increase in syndecan-1 gene expression ($P = 0.001$) in keloid relative to NS, normal skin, as well as HTS (Figure 2b). Despite the increased expression of CD138 at mRNA level in HTS compared with controls, there was no statistically significant difference ($P > 0.05$). Therefore, quantitative protein analysis was also conducted in keloid, HTS, NS, and normal skin. Syndecan-1 protein level was significantly overexpressed ($P = 0.001$) in keloid dermis using anti-human syndecan-1 ELISA and in cultured keloid fibroblasts using the in-cell western blotting technique (see Supplementary Material and Methods for details), relative to NS and normal skin (Figure 2c—e). Although there was a significant increase in the expression of syndecan-1 in HTS and NS tissue compared with normal skin ($P < 0.05$), this could also reflect endogenous syndecan-1 from either immune cells and/or epithelial cells from adnexal structures/sweat glands in HTS and NS dermis, which was also detected in the tissue staining (Supplementary Figures S2—S4).

Syndecan-1 plays an important role as a cell adhesion and growth factor-binding molecule not only during embryogenesis but also during tissue regeneration in mature tissues. Overexpression of syndecan-1 in basal keratinocytes promotes proliferation during epidermal development, but restricts proliferation during wound healing (Stepp et al., 2015). Currently, the mechanism behind high expression of syndecan-1 in epidermis across all the tissue samples used in this study remains unclear. Based on increasing insight into the role of syndecan-1 in the pathobiology of fibrotic diseases and cancer, to our knowledge previously unreported, our findings encourage one to follow up, next, whether syndecan-1 is functionally involved in the progression of fibrogenesis and the maintenance of chronic, fibrogenic inflammation in KS (Bagabir et al., 2012). It has previously been shown that microRNA-143 is downregulated in melanoma and negatively regulates overexpressed syndecan-1 (Li et al., 2014). Interestingly, a comparative study of microRNA profiling in fibroblast also showed that microRNA-143 is downregulated in keloid (Li et al., 2013). This may be investigated most instructively in the KS organ culture, using previously published in situ gene knockdown techniques (Syed et al., 2013). The distinction between DFSP tumor and scar tissue surrounding the tumor in the DFSP sample is quite interesting in terms of syndecan-1 expression and needs further elucidation. Histologically, scar tissue consists of fascicles of fibroblasts/myofibroblasts that have relatively plump nuclei as opposed to the slender nuclei of DFSP. Moreover, the fascicles in scar tissue are longer and have horizontal orientation as opposed to DFSPs that in typical cases have short fascicles arranged in a prominent storiform pattern. CD34 also helps to distinguish scar tissue from DFSPs. The former is generally negative, whereas DFSPs show strong and diffuse staining. Furthermore, the lesional cells that constitute DFSPs are fibrohistiocytic cells or cells related to dermal dendritic cells and not pure fibroblasts, whereas in scar tissue surrounding to DFSP tumor may have a keloid-like environment or may have mainly myofibroblasts, hence the difference in expression of CD138, which need to be further investigated in future. In summary, one can use a combination of morphological analysis and CD34 immunostaining to distinguish scar tissue from DFSP (Prieto et al., 1994). It can however be difficult in some cases; therefore, CD138 may add to the ancillary studies used in solving this dilemma.

In conclusion, we have shown, previously unreported, that syndecan-1 is significantly overexpressed in keloid compared with HTS and NS, and completely absent in DFSP. Beyond its potential value as a biomarker, to our knowledge previously unreported, to distinguish between keloid and HTS, syndecan-1 can also be used as a vital diagnostic marker to distinguish misdiagnosis between keloid and DFSP, as it has been clearly shown in the tumor tissue samples in this study and in the previous studies, corroborating our findings, that DFSPs do not express syndecan-1 in dermis (O'Connell et al., 2004; Orosz and Kopper, 2000).

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