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Letter to the Editor

Quantitative histological analyses and transcriptional profiling reveal structural and molecular changes of the dermal extracellular matrix in cellulite

Cellulite is a chronic skin condition that is characterized by dimpling and lumpiness of the skin on the thighs, hips and buttocks (Fig. 1a). It has been suggested that the incidence of cellulite correlates with the body mass index (BMI) [1] but also women with a low/normal BMI (BMI: 18–25) frequently show the appearance of cellulite [2]. Thus, cellulite is not simply caused by overweight or obesity, but other, thus far unknown factors apparently contribute to its pathogenesis. Since the exact mechanisms and causes of cellulite formation have not yet been determined, there are at present a large number of conflicting hypotheses [3,4].

We first performed the largest ever histological and immunohistological studies in 25 females with cellulite, 19 females without cellulite, and five males without cellulite. Hematoxylin stains revealed that epidermal rete ridges were shallower in cellulite skin than in female skin without cellulite (Fig. 1b, e and f), and that the papillary dermis was consistently thinner in cellulite skin (Fig. 1c). Elastica van Gieson stains identified an abundant elastic fiber network in the dermis of female control skin, whereas elastic fibers were scarce and disorganized in cellulite skin (Fig. 1d). In six out of 25 cellulite specimens, but not in normal skin, areas of irregularly condensed collagen were observed, associated with absence of a well defined papillary dermis (Supplementary Fig. 1a and b). In normal skin, there was a clear distinction between subcutaneous adipose tissue and overlying dermis. In contrast, the subcutaneous adipose tissue drastically protruded into the dermis of cellulite skin (Fig. 1g), in agreement with observations obtained by noninvasive MRI [2,5]. We found a significantly greater amount of protruded adipose tissue in cellulite skin (10.5% ± 1.1%, n = 24) than in female control skin (5.0% ± 0.9%, n = 18; p = 0.001) or male control skin (3.9% ± 1.1%, n = 4; p = 0.03) (Fig. 1h).

We next investigated the mRNA expression levels of major extracellular matrix-related genes. We found that the mRNA expression levels of the type I collagen α1 (I) chain (COL1A1), COL3A1 and COL12A1 were not significantly different between cellulite and female control skin (Supplementary Fig. 2). The expression levels of elastin, fibrillin, fibulin-5, LOX and LOXL1 were comparable cellulite and female control skin (Supplementary Fig. 2). Analysis of the expression levels of MMPs and TIMPs revealed that expression of MMP1 was significantly decreased (P = 0.007) in cellulite skin and that there was a slight reduction of MMP3 expression (P = 0.09) (Supplementary Fig. 2).

We next investigated the global transcriptional profiles of dermal fibroblasts in cellulite skin. Gene expression analysis revealed several differentially expressed genes in fibroblasts obtained by laser capture microdissection of cellulite skin (Supplementary Table). Due to their potential roles in extracellular matrix production, the protein levels of sarcoglycan-gamma (SGCG), biglycan (BGN) and EFEMP1 were also examined. In normal skin, biglycan was mainly detected in the papillary dermis, with the strongest expression immediately below the epidermis (Fig. 2a). By contrast, biglycan expression was greatly reduced in the dermis of cellulite skin (Fig. 2a). The biglycan expression scores were significantly lower in cellulite skin than in female control skin (P = 0.006) (Fig. 2c). Immunohistochemical analyses revealed expression of sarcoglycan-gamma in epidermal keratinocytes and dermal fibroblasts, as well as in smooth muscle cells of the arrector pili muscle (Fig. 2d). Expression of sarcoglycan-gamma was decreased in cellulite samples compared with female control skin, in particular in papillary dermal fibroblasts (Fig. 2d). EFEMP1 contributes to the integrity of elastic fibers in the connective tissue of fasciae and the vaginal wall, and lack of EFEMP1 causes inguinal hernias and pelvic prolapse [6,7]. Immunohistochemical analyses of normal skin showed expression of EFEMP1 in the suprabasal layers of the epidermis and also co-localization with oxytalan and elastic fibers in the dermis (Fig. 2e and f). Decreased EFEMP1 expression in this area was observed in cellulite samples, and oxytalan and elastic fibers were also decreased in cellulite samples (Fig. 2e and f). In fibrous septa within normal subcutaneous tissue, where a dense and robust elastic fiber network was observed, EFEMP1 largely co-localized with elastic fibers (Fig. 2g). In cellulite samples, EFEMP1 expression was reduced (Fig. 2g). We next investigated EFEMP1 expression levels by quantitative real-time RT-PCR with RNA isolated from whole skin lysates. Expression of EFEMP1 was significantly down-regulated in cellulite skin (fold change: 0.72, P = 0.023) (Fig. 2h).

The results of our histological study indicate that shallower rete ridges, a thinner papillary dermis, irregularly condensed collagen, disorganized elastic fibers, and greater amounts of protruded adipose tissue are key features of cellulite. A key finding was the increased protrusion of adipose tissue into the dermis. Together with the reduction of elastic fibers and the thinning of the papillary dermis, this argues for a reduced function of the dermis as a cushion against fat protrusion, leading to the outward manifestation of ripples in the skin, a characteristic feature of cellulite. In some cellulite specimens, in which the papillary dermis was greatly diminished, we observed irregularly condensed collagen fibers, likely creating uneven physical properties in the skin. How these observations relate to the formation of cellulite will require further study. However, we found that the expression of MMP1 and MMP3 was significantly decreased in cellulite samples compared with female control samples. Since MMP1 and MMP3 are major

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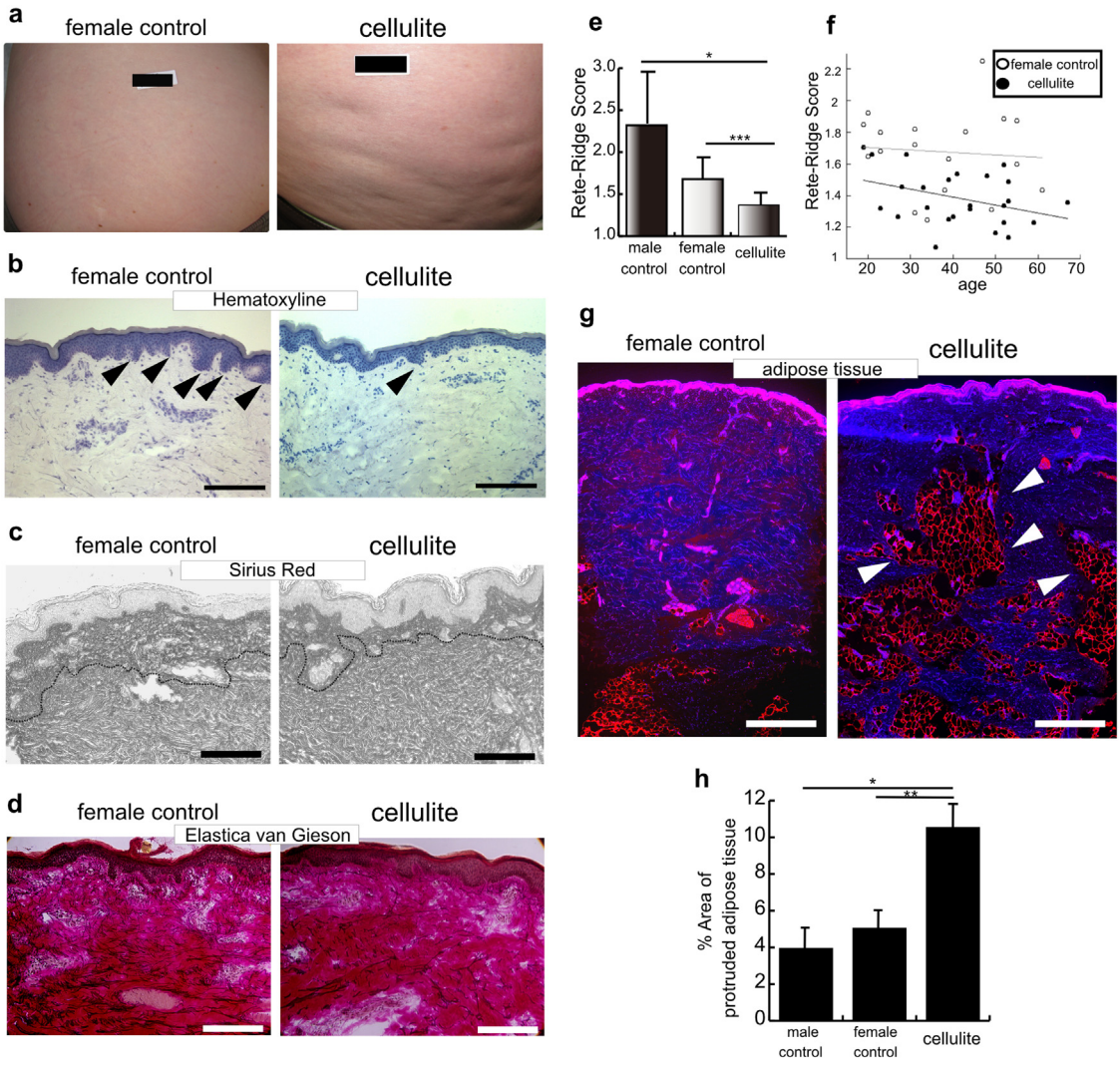

Fig. 1. Comparison of cellulite and control skin samples Photographs of the buttock skin of female control and cellulite skin (a). Representative images of hematoxylin stained sections from female control and cellulite samples (b). Well-formed rete ridge structures are indicated by arrowheads. Sirius red (collagen) stained sections from female controls and cellulite samples (c). Representative black & white images of sections are shown. Sirius red staining revealed that the total amount of collagen in the entire dermis was not consistently different in cellulite and female control skin (c). Two distinct dermal layers, namely the upper papillary and the lower reticular dermis, could clearly be distinguished, based on the different arrangement and amount of collagen fibers. The black dashed line represents the boundary between papillary and reticular dermis. Note that the papillary dermis in the cellulite sample is thinner than in control skin (c). (d) Elastica van Gieson stained sections represent elastic fibers (black) and collagen (red). Representative images of sections of female controls and cellulite samples are shown. Disorganized elastic fibers are observed in the dermis of the cellulite sample. Bars: 200 μm (b–d). (e) The rete ridge score was calculated to assess the frequency and depth of rete ridge structures. Score values are expressed as mean ± SEM calculated from cellulite (n = 24), female control (n = 19), and male control samples (n = 5).
***$P < 0.001$. The rete ridge score was significantly lower in cellulite skin (score 1.38) than in female control skin (score 1.68; $p \leq 0.001$) or in male control skin (score 2.32; $p \leq 0.05$). Since flattening of epidermal rete ridges might be associated with aging, rete ridge scores were plotted against age for each cellulite and female control skin sample (f). Regression lines were drawn separately for each group. While there was a trend for reduced rete ridge scores with aging in normal skin (r=−0.005) and in cellulite skin (r=−0.0016), rete ridges were shallower in cellulite than in female control skin for each age group (f). (g) Immunohistochemical stainings for perilipin, a major adipocyte-specific phosphoprotein to reveal adipose tissue (red). Co-staining of nuclei was performed using Hoechst 33342. Representative images of female control and cellulite samples are shown. Arrowheads indicate protruded adipose tissue in the dermis. Bars: 1 mm. (h) The area of protruded adipose tissue was measured in a 4-mm-deep and 3-mm-wide area (below the epidermis) to quantify the differences in the amount of protruded adipose tissue. The percentages represent the relative area covered by perilipin-positive adipose tissue and are expressed as mean ± SEM calculated for cellulite (n = 24), female control (n = 18) and male control (n = 4) samples. ** $P < 0.01$, * $P < 0.05$.

collagenases of the dermis, decreased MMP1 and MMP3 levels may contribute to the observed irregularly condensed collagen.

Our findings indicate that decreased EFEMP1 expression might contribute to the adipose tissue protrusion in cellulite, also known as 'fat herniation' due to less elastic fibrous septa that can no longer function as partition. An altered orientation and uneven thickness of the fibrous septa indeed represent characteristic features of cellulite [5,8]. Taken together, our analyses identified characteristic structural and molecular features of the dermis in cellulite, indicating a more comprehensive involvement of the skin that goes beyond the established alterations of the subcutaneous adipose tissue. The detailed mechanisms how the identified extracellular matrix-associated proteins might contribute to cellulite pathogenesis, as well as their relevance as potential therapeutic targets will need to be investigated in future studies.

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