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RESEARCH ARTICLE

Deregulation of versican and elastin binding protein in solar elastosis

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Abstract Several changes in skin appearance including loss of elasticity and wrinkle formation are associated with alterations in the composition of the dermal extracellular matrix. They are induced by intrinsic aging or by environmental factors such as UV light referred to as photoaging. A general characteristic in the histology of photoaged skin is the accumulation of elastotic material suggesting impaired formation and/or massive breakdown of elastic fibres. In order to shed light on some of the underlying mechanisms we tracked two of the major players in elastic fibre formation in different skin conditions: EBP (elastin binding protein), a regulator of elastic fibre assembly and VER (versican), a component of functional elastic fibres as well as non-functional elastotic material. Using quantitative RT-PCR on skin biopsies we found that the expression levels of VER and EBP were unaltered during intrinsic skin aging. Upon acute UV stress however, VER and EBP showed different regulation patterns: VER mRNA increased after 6 h and was further up-regulated until 24 h. The EBP mRNA by contrast was reduced after 6 h but showed massive induction at 24 h after acute UV stress. In chronically sun-exposed skin, VER protein was accumulated similar to elastotic material in the extracellular space, whereas its mRNA level was consistently reduced compared to sun-protected skin. The EBP mRNA by contrast showed slightly increased expression levels in the sun-exposed area compared to its sun-protected counterpart. Based on these data we propose a model which may help to explain parts of the mechanisms leading to the formation of elastotic masses. We further hypothesize that the presence of elastotic material triggers some yet unknown feedback mechanism(s) resulting in altered expression patterns of VER and EBP in chronically sun-exposed skin.

Keywords Skin aging · Solar elastosis · Elastic fibres · Elastogenesis · Elastin binding protein · Versican

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Introduction

Skin is the largest organ of the human body and like all other organs it undergoes chronological/intrinsic aging. Unlike other organs skin is exposed to environmental damage which is superimposed on chronological aging. UV irradiation from the sun is the major factor of extrinsic aging, thus mostly referred to as photoaging. It induces dramatic

changes in the mechanical properties, morphology and physiology of the skin. The mechanical properties are mainly determined by the dermal extracellular matrix (ECM). Its major components are the collagen fibres conferring tensile strength and the elastic fibres giving resilience. Besides the collagens and elastic fibres proteoglycans serve important functions in interconnecting multiple binding partners to form higher order networks.

With photoaging, the fibrillar collagens type I and III have been shown to decrease in the dermal compartment (Chen et al. 1986; Oikarinen and Kallioinen 1989; Schwartz et al. 1993), whereas elastin accumulates together with other components of the ECM to a largely unstructured material referred to as solar elastosis (Chen et al. 1986; Schwartz et al. 1993). The structural changes observed in the dermal ECM are thought to be in large part responsible for the typical leathery appearance of photoaged skin with deep wrinkles and loss of elasticity.

Versican is a proteoglycan isolated from human skin in large amounts (Carrino et al. 2000). It has been shown to co-localize with elastic fibres (Bernstein et al. 1995; Zimmermann et al. 1994) in the dermal compartment and to be a substantial component of the elastotic material found in photoaged skin (Bernstein et al. 1995). Versican consists of an amino-terminal globular domain (G1) that binds hyaluronan (HA), a central extended region with attached glycosaminoglycans (GAGs) and a carboxy-terminal selectin-like domain (G3) that binds to other matrix components including fibulin-1 (Aspberg et al. 1999), fibulin-2 (Olin et al. 2001) and fibrillin-1 (Isogai et al. 2002; Zimmermann and Ruoslahti 1989). The GAGs attached to the central region are of the chondroitin sulphate (CS) type and the mRNA that codes the GAG attachment region can undergo differential splicing to produce four variants: V0 with two (α and β) GAG binding regions, V1 containing only the β GAG exon, V2 with the α GAG exon alone and V3 without any GAG exon (Dours-Zimmermann and Zimmermann 1994; Ito et al. 1995; Zako et al. 1995). Thus, V3 is formed by the G1 and G3 domains only and is therefore predicted to be a glycoprotein and not a proteoglycan. Several studies and observations point to an important relationship between the larger chondroitin sulphate containing versican variant V1 and the small chondroitin sulphate lacking variant V3 in elastin production and assembly of elastic fibres (Faris et al. 1992; Lemire et al. 1994; Lemire et al. 1996; Wight et al. 1997).

The extracellular assembly of tropoelastin into mature elastic fibres on the microfibrillar scaffold is mediated by a cell surface protein referred to as elastin binding protein (EBP). This protein binds tropoelastin intracellularly and escorts it through the secretory pathways thereby protecting this highly hydrophobic protein from premature self-aggregation and proteolytic degradation (Hinek and Rabinovitch 1994). At the cell surface EBP binds tropoelastin, the cell membrane and galactosugars via three separate sites (Hinek et al. 1988; Mecham et al. 1989). The influence of galactosugars is crucial for the coordinated release of both tropoelastin and EBP from the cell surface (Hinek et al. 1991; Hinek et al. 1988). Large amounts of galactosugars surrounding the cells have been shown to displace EBP from the cell surface (Hinek et al. 1991) resulting in impaired elastic fibre assembly (Hinek et al. 1992).

Therefore, versican as a structural component of both, functional elastic fibres as well as non-functional elastotic material and EBP as a regulator of elastic fibre formation were subject to our investigation of expression profiles in sun-protected and photoaged adult human skin. Our data suggest that the accumulation of versican in solar elastosis triggers a negative feedback loop resulting in reduced versican and increased EBP mRNA levels in photoaged skin which leads to sustained impairment of elastic fibre formation.

Materials and methods

Skin samples

For the investigation of expression levels during intrinsic aging and after acute UV stress skin samples from a clinical study approved by the Ethics Committee of the Medical Association of Hamburg (OB/4/02) were used. All participants signed informed consent forms before their inclusion in the study. Full-thickness skin biopsies were obtained from five young (25–35 years) and five aged volunteers (60–68 years). Sun-protected buttock skin showing no clinical evidence of photo-damage was irradiated with an individually determined single dose of two

minimal erythemal dose (MED) of solar simulated irradiation (SSR, Solar Sun Simulator SU 5000, MUT, Wedel, Germany) according to the COLIPA guidelines (European Cosmetic, Toiletry and Perfumery Industry, Brussels, Belgium). The spectrum of solar simulated light consisted of 6.6% UV-B and 93.4% UV-A (80.6% UV-A1 and 19.4% UV-A2). The irradiation intensity of solar simulators was determined with an IL-1700 radiometer (International Light, Newburyport, MA, USA). Full-thickness 5 mm punch biopsies were taken from the non-irradiated site as well as from the irradiated sites after 6 ($n = 5$), 24 ($n = 10$) and 72 ($n = 5$) h. Immediately after removal, punch biopsies were snap-frozen in liquid nitrogen and stored until further use.

For the analysis of chronically sun-exposed (face) versus sun-protected (lateral upper arm) aged skin (64–91 years), samples were collected from routine dissection course material of five donors (Department of Anatomy, Christian Albrecht University Kiel, Kiel, Germany). Skin samples for real-time PCR studies were collected within 24 h after death using a sterile 8 mm biopsy punch (Stiefel Laboratorium, Offenbach, Germany), immediately frozen in liquid nitrogen and stored at −80°C until analysis

Extraction of RNA from human biopsies

For RNA extraction from full-thickness skin biopsies, frozen samples were homogenized in RNAzol® with an Ultra Turrax T8 (IKA, Staufen, Germany) and further processed following the manufacturer's protocol.

cDNA Synthesis and quantitative RT-PCR

For cDNA synthesis the High Capacity cDNA Archive Kit (Applied Biosystems, Forster City, US) was used according to the supplier's recommendations. The resulting cDNA was analyzed by Real-Time TaqMan®-PCR using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Forster City, US). FAM labelled primers for the RT-PCR were distributed by Applied Biosystems and are as follows: Inventoried TaqMan Assays for 18 S rRNA (Hs99999901_s1), EBP (Hs01035164_m1), total versican (Hs00171642_m1), V1 (Hs01007937_m1) and V3 (Hs01007941_m1). PCR conditions were as follows: 50°C for 2 min, 95°C for 10 min followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. Real-time PCR data were analyzed using the Sequence detector version 1.6 software included with the 7700 Sequence Detector and GraphPad Prism 4.0 software. In order to show individual variations of the donors, delta CT values (dCT) were generated according to (Livak and Schmittgen 2001) using 18 s rRNA as internal control and are shown as 1/dCT values in Figs. 1, 2 and 5. Fold regulation of expression levels was determined with the $2^{-\mathrm{ddCT}}$ method (Livak and Schmittgen 2001) using the non-irradiated control sample as calibrator (Fig. 3). Where indicated *P*-values were determined on $n = 10$ samples using the paired T-test and GraphPad Prism 4.0 software.

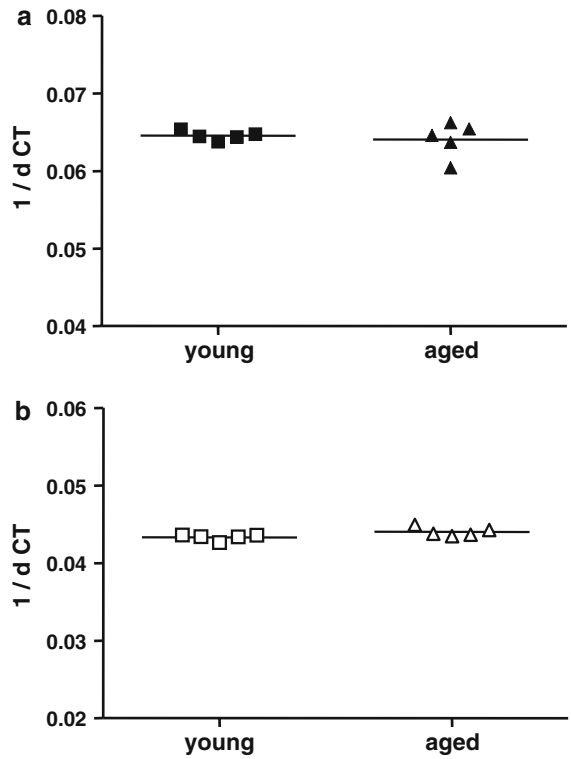

Fig. 1 Versican and EBP expression levels are not affected by intrinsic aging. Quantitative RT-PCR was used to assess the RNA expression levels of versican (**a**) and EBP (**b**) in skin samples from a sun-protected area obtained from five young (25–35 years) and aged (60–68 years) donors. Values are shown as 1/dCT for each individual using 18 s rRNA as endogenous control

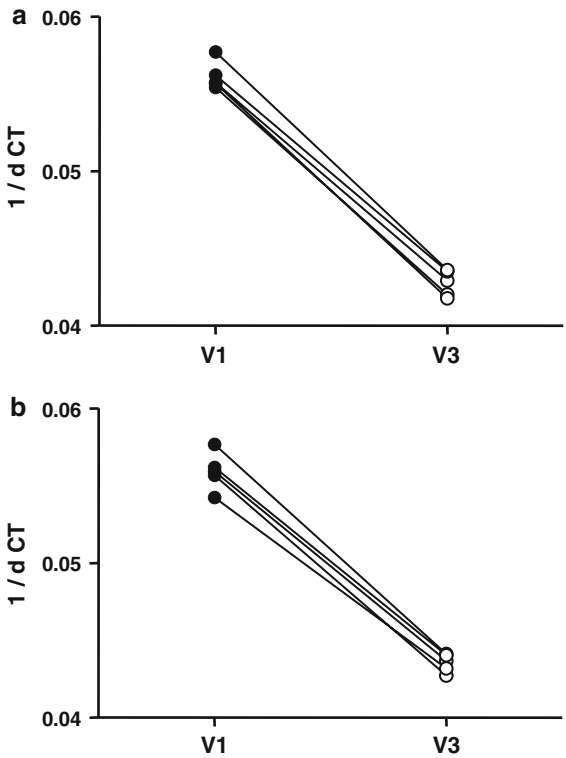

Fig. 2 The versican expression level in young and aged human skin is largely represented by the V1 variant. Using quantitative RT-PCR, the mRNA levels of the larger isoform V1 were compared to the shortest isoform V3 in sun-protected skin of five young (**a**) and five aged (**b**) donors. Values are shown as 1/dCT for each individual using 18 s rRNA as endogenous control

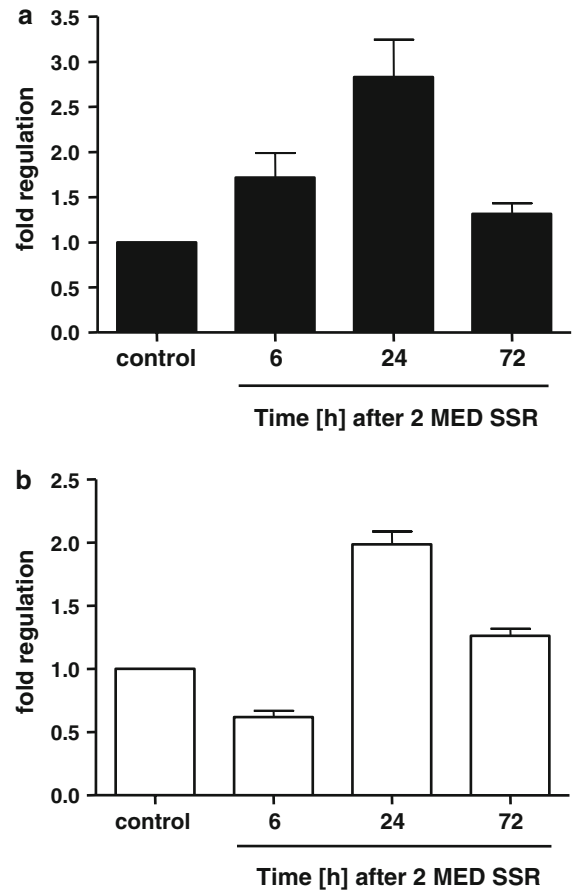

Fig. 3 Versican and EBP mRNA levels are differentially regulated after acute UV stress. The RNA expression levels of versican (**a**) and EBP (**b**) were assessed in skin samples (combinations of young and aged donors) of a sun-protected area without (control, $n = 10$) and 6 h ($n = 5$), 24 h ($n = 10$) and 72 h ($n = 10$) after irradiation with 2 minimal erythemal doses of solar simulated radiation (2 MED SSR). Using 18 s rRNA as endogenous control, 1/dCT values were generated and mean values with the respective standard deviations are shown relative to the respective non-irradiated sample

Immunohistochemistry

Skin samples were fixed in 10% neutral buffered formalin, embedded in paraffin using routine procedures, sliced at 7 μm thickness, and mounted on glass slides. Prior to staining, paraffin sections were deparaffinized with xylene, rehydrated, and washed three times with phosphate-buffered saline (PBS). Immunohistochemical staining was performed using the avidin–biotin–peroxidase complex technique (Vectarstaing Kit, Linaris). The tissue sections were incubated for 30 min in 3% hydrogen peroxide at room temperature to inhibit the endogenous peroxidase before the staining procedure. Following the instructions, the sections were blocked with 1% bovine serum albumin in PBS for 1 h and next incubated with a primary monoclonal anti-versican antibody (US Biological, dilution 1: 1000) for 24 h at 4°C followed by biotinylated-avidin secondary antibody treatment. The colour was developed with the diaminobenzine substrate method. After washing and colour detection, the sections were counterstained with haematoxylin and mounted in Aquatex® (Merck, Darmstadt, Germany). The results of the immunostaining were visualized and digitally photographed using a Zeiss Axiovert microscope equipped with a digital camera (Axiocam, Zeiss, Jena, Germany).

Histology

To determine general morphology, serial sections were stained with hematoxylin-eosin (HE) stain and for determination of elastic fibers, Weigert's resorcin-fuchsin staining (Romeis 1989) was used.

Results

Expression levels of Versican and EBP are unaltered during intrinsic skin aging

The versican protein is predominantly found in the dermal compartment of human skin (Zimmermann et al. 1994). Using quantitative RT-PCR we found that versican mRNA levels were 100 times higher in the dermis compared to the epidermis (data not shown). In line with the literature, this indicates that the amount of versican mRNA detected in total skin biopsies largely represents the situation in the dermal compartment and thus total biopsies were used in all following experiments.

In order to investigate the expression patterns of versican and EBP during skin aging we first determined their mRNA levels in normal human sun-protected skin of young (25–35 years) and aged (60–68 years) adults (Fig. 1). We consistently detected versican mRNA in all subjects without strong individual variation. The expression level was found to be similar in the young and aged subjects (Fig. 1a). The EBP mRNA was expressed at a lower level compared to versican but also with minimal individual variation and equal expression levels in both age groups (Fig. 1b). These data indicate that mRNA expression levels of versican and EBP are largely unaltered during intrinsic skin aging.

V1 mRNA is the predominant versican isoform in human skin

Four different splice variants have been described for versican: V0, V1, V2 and V3. They are not only differentially expressed in various tissues but also seem to serve specific functions. In order to characterize the expression profiles of the different variants in adult human skin we used specific primers for quantitative RT-PCR. The V2 mRNA was below and the V0 level was very close to the detection limit of our assays (data not shown), but V1 and V3 were readily detectable in adult human skin in both young (Fig. 2a) and aged (Fig. 2b) samples. The V1 variant was expressed at a much higher level compared to V3 with an extremely high consistency in all subjects. The difference shown as 1/dCT-values in Fig. 2 represents a difference in copy number of approximately 32-fold for both age groups. Thus, V1 the versican variant containing the β GAG exon with multiple GAG attachment sites, appears to be the predominant versican isoform in adult human skin over V3, the shortest isoform without any GAG chains. In further experiments primers detecting total versican mRNA were used to largely represent the V1 level in this tissue.

Acute UV stress on human skin regulates versican and EBP mRNA expression differentially

In order to investigate the involvement of versican in extrinsic aging, we assessed mRNA expression levels in skin biopsies taken from a sun-protected area 6 h, 24 h and 72 h after exposure to 2 MED SSR and an equivalent non-exposed control of young and aged donors. The mean versican mRNA level increased by 1.7-fold after 6 h of acute UV stress and reached statistical significance ($P < 0.0001$) at the maximum increase of 2.9-fold after 24 h. At the 72 h timepoint, versican expression returned to 1.3-fold over baseline level (Fig. 3a). The mRNA increase was independent of the age group as indicated by the low standard deviations in these samples at all the time points investigated. Thus, the acute stress response of human skin to SSR includes an up regulation of total versican mRNA levels.

By contrast, the EBP mRNA level in the same samples was reduced to 0.6-fold compared to the control sample after 6 h of exposure to 2 MED SSR (Fig. 3b). However, after 24 h the EBP mRNA increased significantly ($P < 0.0001$) by 2-fold and returned to almost baseline level after 72 h. Thus, EBP and versican mRNA are strictly and specifically regulated with time after acute UV stress in human skin.

Versican protein is accumulated together with elastotic material in chronically sun-exposed skin

The mRNA expression level of versican was unaltered in sun-protected skin comparing young and aged samples (Fig. 1), whereas acute UV stress

increased versican expression dramatically (Fig. 3a). In order to investigate long-term effects of sun exposure on the deposition of versican protein in the extracellular matrix we compared skin biopsies from a largely sun-protected area (upper arm) to a sun exposed-area (face) in aged volunteers. Figure 4 shows the results of one representative out of five subjects using an anti-versican antibody on skin sections (Fig. 4a). In line with the literature the staining was largely restricted to the dermal compartment in both areas and was much more intense in the sun-exposed sample. The staining pattern is similar to that obtained by resorcin staining which indicates elastic fibres and showed strong accumulation of elastotic material in the sun-exposed area (Fig. 4b). Thus, versican protein accumulates in the dermis of chronically sun-exposed areas similar as or together with elastotic material.

Chronically sun-exposed skin shows reduced versican but increased EBP mRNA levels

Intrinsic aging did not change the versican mRNA expression level in human skin (Fig. 1), but protein levels in the extracellular matrix of photoaged skin were much higher compared to intrinsically aged skin of the same individuals (Fig. 4). We determined the expression levels of total versican mRNA in the same sun-exposed versus sun-protected samples and found, that it was consistently reduced in the photoaged area of all subjects (Fig. 5a). The 1/dCT values shown in Fig. 5a represent an average decrease in copy number of 1.9-fold in the sun-exposed versus sun-protected area, suggesting, that the accumulation of versican protein found in the extracellular matrix of photoaged skin may trigger a negative feedback loop and reduce further production of the protein.

Conversely, the expression level of EBP was consistently increased in the photoaged area of all subjects (Fig. 5b). The 1/dCT values shown in Fig. 5b represent an average increase in copy number of 1.7-fold, suggesting that UV induced rearrangements of the extracellular matrix may trigger signals that induce enhanced EBP production.

Discussion

Aging processes in connective tissues are in large part characterized by extensive disorganisation of the

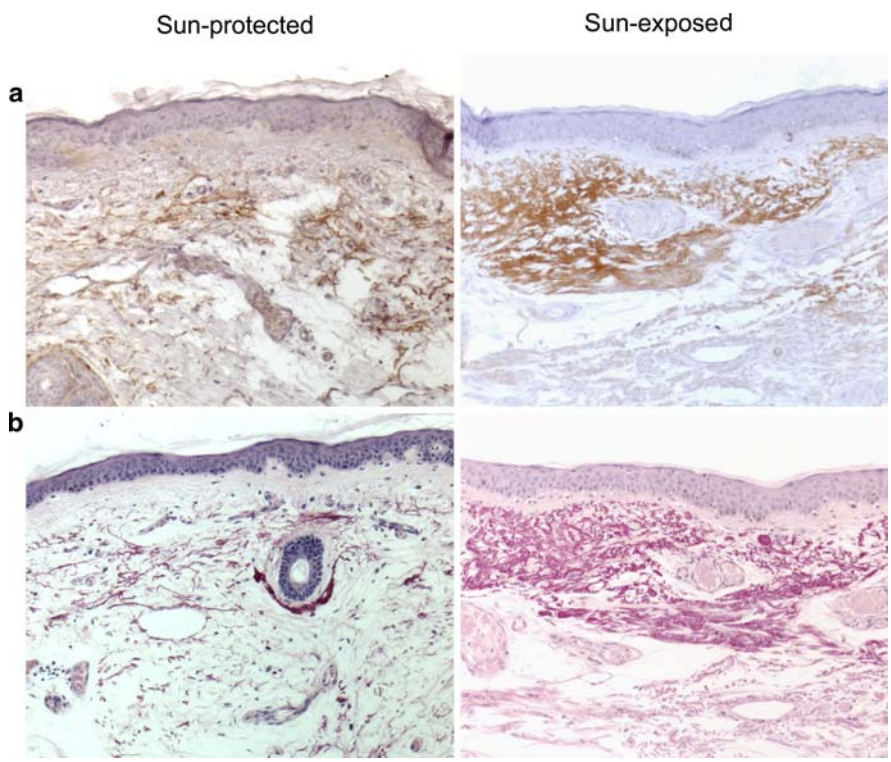

Fig. 4 Versican protein accumulates in chronically sun-exposed skin similar to elastotic material. Skin samples from a sun-protected and a sun-exposed area of aged (64–91 years) volunteers were subjected to histological analysis using a monoclonal anti-versican antibody (**a**) or Resorcin-Fuchsin stain (**b**). One representative out of five individuals is shown

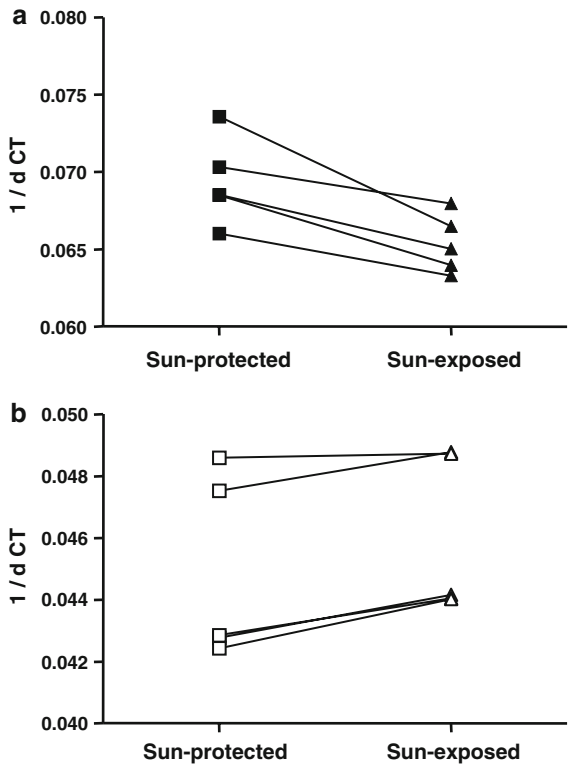

Fig. 5 Expression patterns of versican and EBP are altered in solar elastosis. The expression levels of versican (**a**) and EBP (**b**) were assessed in skin samples of a sun-protected and a sun-exposed area in five aged donors. The values are shown for each individual as 1/dCT using 18 s rRNA as endogenous control

extracellular matrix. The physical properties of skin change dramatically as a function of age (Daly and Odland 1979), and there are also changes in the amount of macromolecule-associated water in the dermis (Richard et al. 1993). Because proteoglycans affect the physical properties of tissues and are involved in hydration and resiliency (Wight et al. 1991), the age-related changes in skin proteoglycans may contribute to the physical properties of aged skin (Carrino et al. 2000). Versican a prominent proteoglycan in adult human skin is found in both functional elastic fibres (Carrino et al. 2000; Sorrell et al. 1999) as well as non-functional elastotic material (Bernstein et al. 1995), but its specific roles remain obscure. EBP is crucial for elastic fibre formation as it is a regulator of tropoelastin assembly on the cell surface (Hinek and Rabinovitch 1994). Therefore we investigated the expression patterns of versican and EBP in adult human skin in relation to intrinsic and extrinsic aging in order to unravel their involvement in decline of skin firmness and function with age.

In previous studies on human skin the versican protein was found in the basal layer of the epidermis, however predominantly in the dermal compartment (Zimmermann et al. 1994). Similar to these findings we detected total versican mRNA in human epidermis at a 100-fold lower level than in the dermis, confirming the previous data on the mRNA level (data not shown). Thus, expression levels detected in total human skin were used to represent largely the amount of mRNA in the dermal compartment.

Fetal skin contains a significantly higher proportion of versican and HA than adult skin (Sorrell et al. 1999) and these matrix components are thought to support the active cellular proliferation, migration and differentiation events that are required for growth and development (Knudson and Knudson 1993). Comparing different age groups ranging from fetal to adult and senescent stages, changes in content and quality of the versican protein have been found including a decrease in versican protein with increasing age (Carrino et al. 2000; Sorrell et al. 1999). In our comparison of total versican mRNA levels in sun-protected skin of two different age groups (25–35 years versus 60–68 years) we could not detect any decline in expression with age (Fig. 1a). In contrast, the expression levels of both versican and EBP showed extremely low individual variations and were equal in both age groups. This suggests that the differences found in versican protein with age (Carrino et al. 2000; Sorrell et al. 1999) are due to some post-transcriptional mechanisms and skin appearance characteristic for intrinsic aging (sagging and fine wrinkling with reduced elastic recoil (Montagna et al. 1989)) is not related to changes in versican or EBP mRNA levels.

The four versican variants which may be produced by alternative splicing are differentially expressed in different tissues with V1 being the most widespread and the predominant in terms of expression levels (Cattaruzza et al. 2002). Similar to these findings V2 was not detectable and V0 was very close to the detection limit (data not shown) in our experiments using quantitative RT-PCR. V3 expression levels were detectable but still low compared to V1 which was by far the prevalent isoform independent of the age-group investigated (Fig. 2). Thus, expression

levels of total versican mRNA represent roughly the expression pattern of the V1 variant in adult human skin. This protein is expected to carry 12–15 GAG chains (Dours-Zimmermann and Zimmermann 1994) and to bind HA forming pericellular coats that are anti-adhesive and promote proliferation and migration (Evanko et al. 1999). These are functions important in fetal skin for growth and development (Knudson and Knudson 1993) as well as in wound healing to promote regeneration (Oksala et al. 1995; Pierce et al. 1991; Wight et al. 1992).

Acute UV stress in usually sun-protected skin induced rapid induction of total versican mRNA levels which declined to almost baseline level after 72 h (Fig. 3a). High levels of versican have been shown to protect cells from oxidative stress induced apoptosis (Wu et al. 2005) and V1 in particular protects fibroblasts from apoptosis (Sheng et al. 2005). Together these data suggest, that upregulation of versican upon acute UV stress serves to establish a micro-environment promoting an excessive repair and remodelling process with protection of fibroblasts from apoptosis, increased proliferation to ensure tissue repair and water uptake for high mobility of cells including those of the inflammatory response. In contrast, EBP, the major regulator of elastic fibre assembly, shows reduction of its mRNA 6 h after acute UV exposure (Fig. 3b) and massive induction after 24 h. A possible interpretation may be that EBP is not essential to deal with UV damage in the early acute phase. Only later, when structural repair mechanisms take place, it may be needed in larger amounts and thus produced at a higher level (Fig. 3b). The proposed function of EBP in elastic fibre assembly (Hinek et al. 1991) allows an additional assumption: EBP gets in contact with the GAG chains of the microfibrillar scaffold for coordinated release of tropoelastin to the growing elastic fibre (Hinek et al. 1991), but excess galactosugars induce shedding of EBP from the cell surface leading to abnormal fibre formation (Hinek et al. 1991). Thus, the high level expression of versican bearing 12–15 GAG chains in its V1 variant (Dours-Zimmermann and Zimmermann 1994) may induce shedding of EBP from the cell surface (Hinek et al. 1991) which in turn by some yet unknown mechanism may induce increased mRNA expression of EBP.

Comparing chronically sun-exposed to sun-protected areas, we found massive accumulation of versican protein together with elastotic material in the dermis of the sun-exposed area (Fig. 4), confirming previously published data (Bernstein et al. 1995). Interestingly, the versican mRNA levels were reduced in the sun-exposed compared to the sun-protected areas (Fig. 5a). In contrast, early passage fibroblasts established from photoaged skin (Bernstein et al. 1995) showed increased versican expression compared to non-sun-exposed control cultures. This suggests that the extracellular microenvironment in photoaged skin leads to a reduction of versican mRNA expression, but the cells are still able to produce this protein if they are placed in normal culture conditions (Bernstein et al. 1995). In contrast to the reduction of versican mRNA expression in the photoaged area, EBP mRNA expression was higher compared to the sun-protected area (Fig. 5b). Again, based on the proposed function of EBP and its impairment by large amounts of galactosugars in the extracellular space (Hinek et al. 1991), this may be explained in that large amounts of the CS bearing versican protein in the ECM of photoaged skin (Fig. 4b) induces shedding of EBP from the cell surface, signalling the need for reproduction of new EBP protein.

There is increasing evidence that damaged collagen in photoaged skin downregulates type I procollagen synthesis of dermal fibroblasts resulting in general impairment of fibre replacement (Varani et al. 2002; Varani et al. 2004; Varani et al. 2001). Together with the recent finding that the versican protein is enzymatically modified in solar elastosis (Hasegawa et al. 2007), our data suggest, that similar mechanisms might also apply to other components of the ECM like versican.

We therefore propose a hypothetical mechanism of action involving versican and EBP in photoaging which might explain the differences found in acute and chronic UV-exposure of these two components (Fig. 6). Under normal conditions EBP makes contact to the components of the microfibrillar scaffold via their galactosugars for coordinated release of tropoelastin to the growing elastic fibre (1). Upon acute UV stress (2), versican mRNA levels are increased dramatically (3), subsequently leading to high levels of versican protein in the extracellular space (4) providing large amounts of GAG chains. Under these conditions EBP is shed from the cell surface with high frequency and may accumulate together with

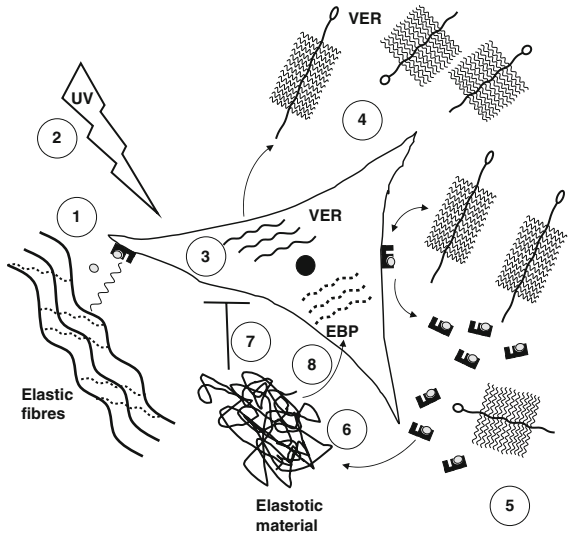

Fig. 6 Illustration of the hypothetical mechanism involving versican and EBP in the formation of elastotic material leading to sustained alteration of expression patterns in chronically sun-exposed skin. Detailed description is given in the text

versican (5) and other components (e.g. tropoelastin) in the extracellular space. As a long term effect of repeated sun exposure, the accumulated and severely damaged material may give rise to solar elastosis (6). The composition of the elastotic material induces by some yet unknown mechanism the sustained reduction of versican mRNA expression (7). Interaction and shedding of EBP on the cell surface leads to a general increase of EBP mRNA levels (8).

We finally conclude that the severe deterioration of the quantitative and qualitative composition of the ECM during photoaging of the skin becomes part of its own regulatory circuit. This results in sustained impairment of ECM function giving rise to the altered physical and functional properties of photoaged skin. Similar mechanisms might also apply to other tissues prone to severe and chronic damage not necessarily caused by UV exposure.

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