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The matricellular protein periostin contributes to proper collagen function and is downregulated during skin aging

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ABSTRACT

Background Periostin is a secreted 90 kDa matricellular protein, which is predominantly expressed in collagen-rich tissues. Collagen is the most abundant protein in mammals and has great tensile strength. Recent investigations have shown that periostin influences collagen fibrillogenesis and biomechanical properties of murine connective tissues.

Objective: We investigated the function of periostin concerning collagen homeostasis during intrinsic and extrinsic skin aging. For this purpose, human skin samples of young and old donors as well as samples of photoaged and sun-protected skin areas were analyzed for periostin expression. Using *in vitro* models, we determined the cell types responsible for periostin expression and performed functional analyses with periostin knockdown cells.

Methods: TaqMan Real-Time PCR, UV irradiation, knockdown experiments, immunostaining, electron microscopy, collagen degradation assay, collagen crosslink analysis.

Results: Periostin expression is highest in the papillary dermis and downregulated during skin aging. Fibroblasts and non-follicular skin derived precursors were identified as main source for periostin expression in human skin. Periostin knockdown in fibroblasts has no effect on collagen expression, but results in an increased fibril diameter and aberrant collagen structure. This leads to an increased susceptibility of collagen toward proteases, whereas recombinant periostin protects collagen fibrils from degradation.

Conclusion: Our data show that periostin plays an important role for proper collagen assembly and homeostasis. During skin aging periostin expression decreases and contributes to the phenotype of aged skin.

1. Introduction

Human skin is continuously exposed to environmental influences and is therefore subjected to both intrinsic as well as extrinsic aging processes. Among all external factors, UV radiation is the main factor for accelerated skin aging. Chronic UV exposure superimposes the intrinsic aging process and thereby increases the saggy and wrinkled appearance of aged skin [1,2]. The extracellular matrix (ECM) in the dermis produced by fibroblasts is composed of a mesh of fibrous proteins, *e.g.* collagen and elastic fibers, and glycosaminoglycans that influence the outer appearance of the skin. With 85–90% of its dry weight collagens predominate in the skin and their structural integrity is essential for its tensile strength. Collagen homeostasis is primarily regulated by dermal fibroblasts that express and secrete collagen, and also internalize degraded collagen. During skin aging, the dermal collagen content decreases and the remaining fibers appear more and more unstructured and fragmented, a phenomenon which in turn impairs the functionality and productivity of dermal fibroblasts [2,3].

In the '90s, a new group of proteins was defined, which are potent mediators of cell–matrix interactions – the matricellular proteins [4]. Matricellular proteins are secreted extracellular proteins that do not exhibit primary structural functions but modulate cell function by interacting with cell-surface receptors, bioactive molecules, and matrix components like collagens [5]. The secreted 90 kDa protein periostin belongs to this group of

Abbreviations: AA2P, L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate; BAPN, β-aminopropionitrile-fumarate; Ct, cycle threshold; DHLNL, dihydroxylysinonorleucine; ECM, extracellular matrix; HDF, human dermal fibroblasts; HHMD, histidinohydroxymerodesmosine; HLNL, hydroxylysinonorleucine; HP, hydroxylysylpyridinoline; HPLC, high performance liquid chromatography; LOX, lysyloxidase; MMP, matrix metalloproteinase; qRT-PCR, quantitative real-time polymerase chain reaction; rh, recombinant human; SKP, skin derived precursor; SSR, solar simulated radiation; TEM, transmission electron microscopy; UV, ultraviolet.

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matricellular proteins. Unlike most matricellular proteins, periostin is not only expressed during embryogenesis or pathogenesis, but also in healthy, adult collagen-rich connective tissues [6]. While periostin could be identified as an important regulator of cell–matrix interaction in heart [7,8], bone [9,10], and wound healing [6,11,12], its function in human skin is mainly unexplored. However, it could be shown in periostin knockout mice that it is important for mechanical stabilization of ECM architecture. Periostin$^{-/-}$ mice exhibit decreased collagen crosslinking [13], impaired wound closure [12], and increased risk of heart failure [8,12,13]. Though, the function of periostin is tissue-specific and varies among induction of collagen expression, linker activity between collagen type I, collagen type V, fibronectin, and tenascin-C [13–15], and influencing different signaling pathways [16]. With regard to collagen type I, periostin was identified in murine calvarial osteoblasts to mediate the activation of lysyloxidase (LOX) [17]. This enzyme is responsible for intermolecular crosslinking of collagen fibrils, and therefore essential for their mechanical stability and tensile strength. Impaired collagen crosslinking is associated with severe connective tissue failures such as cutis laxa syndrome [18] and is also associated with photoaged skin [19]. Furthermore, it is hypothesized that periostin has an effect on collagen fibril assembly by promoting the lateral association of single collagen fibrils [20].

Consequently, we investigated the role of periostin for collagen homeostasis during skin aging. We could show that periostin is downregulated during intrinsic and extrinsic aging processes. *In vitro* experiments with periostin knockdown fibroblasts revealed thickened collagen fibrils and an impaired structure, but no influence on collagen expression. Furthermore, we have evidence that periostin plays a role in regulating the susceptibility of collagen toward proteases such as matrix metalloproteinases (MMP).

2. Materials and methods

2.1. Ethics statement

Human skin punch biopsies were obtained from two clinical studies (PV2992 and 09-154), approved by the ethics committee of medical association of Hamburg and Luebeck, Germany and from routine surgery. All volunteers provided written, informed consent, and samples were collected in accordance with the Declaration of Helsinki Principles. The proceeding was approved and cleared by the institutional ethics review board (Beiersdorf AG, Hamburg, Germany).

2.2. Clinical studies

To analyze periostin expression during intrinsic aging, skin biopsies (6 mm) of the gluteal region of 16 young (21–34 years) and 15 old volunteers (60–70 years) were taken. To determine periostin expression in extrinsically aged skin, punch biopsies (4 mm) of 12 donors (male, 65–84 years) were collected. Skin samples from the neck region (= sun-exposed) were compared with corresponding skin samples from the inner upper arm (= protected). The probands were pre-screened by a dermatologist for signs of solar elastosis in the neck.

2.3. Cell culture

If not further indicated, human dermal fibroblasts (HDF) were obtained *via* outgrowth from skin biopsies from different female donors as previously described [21]. For stable transduction of fibroblasts and analysis of collagen stability neonatal fibroblasts (HDFneo) from male foreskin (Tebu-Bio, Le-Perray-en-Yvelines, France) were used. Cells were cultured at 37 °C, 7% CO_2 in the respective media. If not indicated otherwise, fibroblasts were cultured in DMEM (Invitrogen, Carlsbad, CA) with 10% FCS (PAA, Pasching, Austria), 100 U/mL penicillin, 2 mM glutamine (both Invitrogen, Carlsbad, CA), and used in passages 3–9. Cell sorting of non-follicular skin derived precursors (SKPs) and dermal fibroblasts was performed as previously described [22]. To analyze collagen matrix assembly, fibroblasts were cultured under multilayer conditions (50,000 cells/cm^2) and stimulated to produce collagen with 10 ng/mL TGF-β1 (Peprotech, Hamburg, Germany) and 100 μM L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate (AA2P, Sigma–Aldrich) for 7–14 days. Cell viability was determined with cell proliferation reagent WST-1 (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturers' instructions. Irradiation of cells with solar simulated radiation (SSR) was performed as previously described [3].

2.4. Transfection

The stable transfection of HDFneo with periostin shRNA was conducted with SMARTvector 2.0 lentiviral particles® (Thermo Scientific Dharmacon, Lafayette, USA). For this purpose, 50,000 cells/cm^2 were cultured in standard medium for 24 h. The transfection was performed in antibiotic and serum free DMEM with 8 μg/mL polybrene (Millipore, Billerica, USA) and 5 virus particles per cell. The virus suspension was removed after overnight incubation and replaced by standard medium. After two days, selection of the transduced cells started with 1 μg/mL puromycin dihydrochloride (Invitrogen, Carlsbad, CA). A scrambled vector and untransduced fibroblasts served as controls. Periostin knockdown was verified *via* qRT-PCR and Western blot.

2.5. Gene expression analysis

To determine gene expression, mRNA was isolated from skin biopsies or cell cultures. For RNA isolation the skin biopsies were homogenized in RLT buffer using the RNeasy fibrous tissue kit® (Qiagen GmbH, Hilden, Germany) and the PreCellys® (PeqLab Biotechnologies, Wilmington, DE). For cell cultures the RNeasy mini kit® was used according to the manufacturers' instructions (Qiagen GmbH). For reverse transcription 1 μg of total mRNA was converted using the High capacity cDNA reverse transcription kit® (Applied Biosystems, Carlsbad, CA) according to the manufacturers' instructions. 200 ng of cDNA was analyzed with the respective TaqMan® gene expression assay: POSTN (Hs00170815_m1), Col1A1 (Hs00164004_m1) and 18S rRNA (Hs99999901_m1) for normalization. Gene expression data are presented as $2^{-\Delta Ct} \times 10^6$ values or fold changes, calculated according to the comparative Ct method [23].

2.6. Western blot and cell western

Western blot analysis was performed as previously described [24] with the following primary and secondary antibodies: rabbit-anti-periostin (ab14041, abcam, Cambridge, UK), mouse-anti-α-tubulin (clone DM1A, ab7291, abcam), anti-mouse-IRDye700, and anti-rabbit-IRDye800 (Li-Cor Biosciences, Lincoln NE). Successful transfer and amount of loaded supernatant were verified with PonceauS staining.

Determination of collagen assembly was performed *via* cell western. For it, HDF were cultured under multilayer conditions in a 96 well plate for 1–2 weeks, fixed in methanol for 10 min at −20 °C, and blocked with 1% BSA. Collagen detection was performed with mouse-anti-collagen type I antibody (ab90395, abcam, Cambridge, UK). Donkey-anti-mouse IRDye680, and cell staining with DRAQ5 and Sapphire700 (all Li-Cor Biosciences, Lincoln, NE) were used for