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ORIGINAL RESEARCH

Investigation of age-related decline of microfibril-associated glycoprotein-1 in human skin through immunohistochemistry study

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Abstract: During aging, the reduction of elastic and collagen fibers in dermis can lead to skin atrophy, fragility, and aged appearance, such as increased facial wrinkling and sagging. Microfibril-associated glycoprotein-1 (MAGP-1) is an extracellular matrix protein critical for elastic fiber assembly. It integrates and stabilizes the microfibril and elastin matrix network that helps the skin to endure mechanical stretch and recoil. However, the observation of MAGP-1 during skin aging and its function in the dermis has not been established. To better understand age-related changes in the dermis, we investigated MAGP-1 during skin aging and photoaging, using a combination of in vitro and in vivo studies. Gene expression by microarray was performed using human skin biopsies from young and aged female donors. In addition, immunofluorescence analysis on the MAGP-1 protein was performed in dermal fibroblast cultures and in human skin biopsies. Specific antibodies against MAGP-1 and fibrillin-1 were used to examine protein expression and extracellular matrix structure in the dermis via biopsies from donors of multiple age groups. A reduction of the MAGP-1 gene and protein levels were observed in human skin with increasing age and photoexposure, indicating a loss of the functional MAGP-1 fiber network and a lack of structural support in the dermis. Loss of MAGP-1 around the hair follicle/pore areas was also observed, suggesting a possible correlation between MAGP-1 loss and enlarged pores in aged skin. Our findings demonstrate that a critical "pre-elasticity" component, MAGP-1, declines with aging and photoaging. Such changes may contribute to age-related loss of dermal integrity and perifollicular structural support, which may lead to skin fragility, sagging, and enlarged pores.

Keywords: microfibril-associated glycoprotein-1, aging, elastic fibers, extracellular matrix, immumohistochemistry

Introduction

Cutaneous aging manifests itself as a progressive reduction in both the function and reserve capacity of skin tissues, and the alterations in functional connective tissue, especially elastic fibers in the dermis, results in distensibility and loss of skin elasticity, which may lead to gradual deepening of facial rhytides and increased skin sagging and laxity.[1–3] Microfibril-associated glycoprotein-1 (MAGP-1) is a key extracellular matrix protein. It plays essential roles during elastic fiber assembly by integrating elastin, microfibrils, and proteoglycans together, and helps organize them into a complex dermal structure.[4,5] MAGP-1 has been shown to be a key regulator during bone development and remodeling.[6,7] Its function in maintaining blood vessel integrity was also previously discussed;[8,9] however, the role of MAGP-1 during skin aging has not been understood. As essential dermal matrix structures, elastic fibers and their

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associated proteins are critical to skin's flexibility, resilience, and recoil. Functional elastic fibers decline with aging and sun exposure as a result of a combination of declined synthesis, faulty assembly, and increased degradation, leading to skin fragility, sagging, loss of elasticity, and wrinkles.[3,10] Therefore, elastic fibers and their associated proteins are of interest for understanding cutaneous aging, and they serve as a targeted area for antiaging interventions.

MAGP-1 is an integral component of the extracellular matrix that plays a critical role in the organization of elastic fibers.[11] It has been proposed as a bridging molecule that binds tropoelastin onto the fibrillin-containing microfibrils during elastic fiber assembly.[12,13] It mediates the binding and alignment of matrix components during elastogenesis, and facilitates the assembly of complex matrix structures (Figure 1).[14,15,16] Together with other microfibril components, MAGP-1 forms a "pre-elastic fiber" module for proper elastin deposition and mature fiber formation. Among elastic fiber-associated proteins, elastin and fibrillin-1 have been widely studied;[2,3] however, the function of MAGP-1 during skin aging was not investigated or understood previously.

Given the role of MAGP-1 in elastic fiber formation, organization, and stabilization, we hypothesize that it serves a critical function in maintaining the foundation and architecture of the dermal matrix, especially the elastic matrix. In this study, we investigated the changes of the MAGP-1 gene and protein network during aging and photoaging through a combination of in vitro and in vivo studies.

Materials and methods

Gene microarray study

Gene array was performed using skin biopsies from healthy female donors in different age groups. Ribonucleic acid samples were prepared freshly and subjected to quality analysis. Affymetrix GeneChip® human whole genome U133 Plus 1.0 Array plates were used to perform the gene microarray (Expression Analysis, Inc., Durham, NC, USA). A comparison between the two age groups was performed to identify differentially expressed genes, and Student's *t*-test was used to measure the statistic difference; a *P*-value smaller than 0.05 was considered to be statistically different.

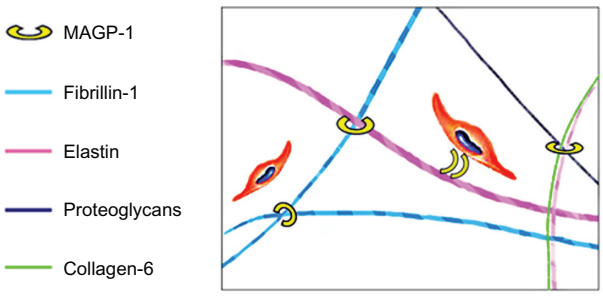

Figure 1 Function of MAGP-1 in human skin.
Notes: Illustration of the role of MAGP-1 in the extracellular matrix assembly. MAGP-1 mediates the binding and alignment of multiple matrix components during elastogenesis, and facilitates the assembly of complex matrix structures.
Abbreviation: MAGP-1, microfibril-associated glycoprotein-1.

UV exposure in vitro

Human dermal fibroblasts from healthy female donors aged 32 years, 38 years, and 39 years (PromoCell GmbH, Heidelberg, Germany; Cell Applications, Inc., San Diego, CA, USA) were grown on supplemented Dulbecco's Modified Eagle's Medium to subconfluence. Cells were subjected to three small doses of ultraviolet (UV) irradiation (UV dose: 10 mJ/cm^2 UVB and 50 mJ/cm^2 UVA) with a 24-hour recovery time between each dose, in order to mimic repetitive exposure to UV. Cells were fixed and subjected to immunocytochemistry studies. After blocking with 1% bovine albumin and 0.5% normal goat serum, goat antihuman MAGP-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) or rabbit antihuman fibrillin-1 (Elastin Products Company, Inc., Owensville, MO, USA) were applied at 1:50 in Tris buffered saline with 1% bovine serum albumin. Experiments were repeated three times, and fluorescent images were captured using the Zeiss Axionskop2 Plus system (Carl Zeiss Meditec AG, Jena, Germany).

Western blot analysis

Cell supernatants were collected from control and UV-irradiated dermal fibroblasts, as described. A total of 10 µL of resuspended supernatant protein sample was loaded onto

Table 1 Age-related changes in key elastic fiber-associated genes from a human full-thickness skin biopsy microarray study

Gene descriptor	Gene symbol	Probe set ID	Fold change	*P*-value
Microfibril-associated glycoprotein 1	*MFAP2* (*MAGP1*)	203417_at	–2.1*	0.023
Microfibrillar-associated protein 4	*MFAP4*	212713_at	–2.7*	0.009
Fibrillin-1 (Marfan syndrome)	*FBN1*	202766_s_at	–5.1*	0.003
Elastin	*ELN*	212670_at	–3.4 (NS)	0.055
Fibulin 2	*FBLN2*	203886_s_at	–3.3*	0.013
Fibulin 5	*FBLN5*	203088_at	–3.1*	0.005
Biglycan	*BGN*	201261_x_at	–3.1*	0.002
Decorin	*DCN*	211813_x_at	–2.9*	0.013

Notes: This table represents differentially expressed elastic fiber-related genes. *Indicates a significant change ($P<0.05$).
Abbreviations: ID, identification; NS, not significant.

each lane of a 4%–20% gradient sodium dodecyl sulfate gel. Primary anti-MAGP-1 was obtained from Novus Biologicals, LLC (Littleton, CO, USA), and was used at 1:1000 dilution. Experiments were repeated three times. Protein band intensity was measured by ImageJ (National Institutes of Health, Bethesda, MD, USA), and the data was expressed as a percentage of control.

Immunohistochemistry

Skin biopsies were collected from the photoexposed areas (forearms) and photoprotected areas (upper underarms or upper thighs) of healthy Caucasian female donors in three different age groups (young, 18–21 years old, n=13; middle-aged, 34–42 years old, n=10; and aged, 57–79 years old, n=12). All protocols were approved by the Greater Delaware Valley Institutional Review Board (Conshohocken, PA, USA), and written informed consent was obtained from all subjects. Biopsies were fixed with neutral buffered formalin; they were then paraffin-embedded and sectioned for immunohistochemical staining. Primary goat antihuman MAGP-1 and antihuman fibrillin-1 (Santa Cruz Biotechnology, Inc.) were applied, followed by Alexa-488 or rhodamine-conjugated secondary antibodies (Vector Laboratories, Inc., Burlingame, CA, USA). Confocal images using 15-micron skin sections were captured by a Leica TCS SP2 system (Leica Microsystems, Wetzlar, Germany), and epifluorescence images were captured by a Zeiss Axionskop2 Plus system (Carl Zeiss Meditec AG).

Results

MAGP-1 is highly expressed in human skin dermis and colocalizes with fibrillin-1

Confocal imaging of MAGP-1 in horizontal skin sections demonstrated that this protein was highly expressed in dermal tissue from young donors (Figure 2A; green fluorescence). This protein forms fibril-like structures with high density in the dermis, and wraps around hair follicles (Figure 2A; arrows). Similar to fibrillin-1 (a key microfibril component), MAGP-1 condensed at the papillary dermal region, connecting epidermis and papillary dermis (Figure 2B). Both proteins were visualized in human skin biopsies using immunofluorescence staining (green, MAGP-1; red, fibrillin-1). Colocalization of MAGP-1 with fibrillin-1 oxytalan fibers underneath the dermal–epidermal junction region was observed (Figure 2B, merged in yellow; the figures that are shown are representative images of the skin biopsy samples from multiple donors, n=13).

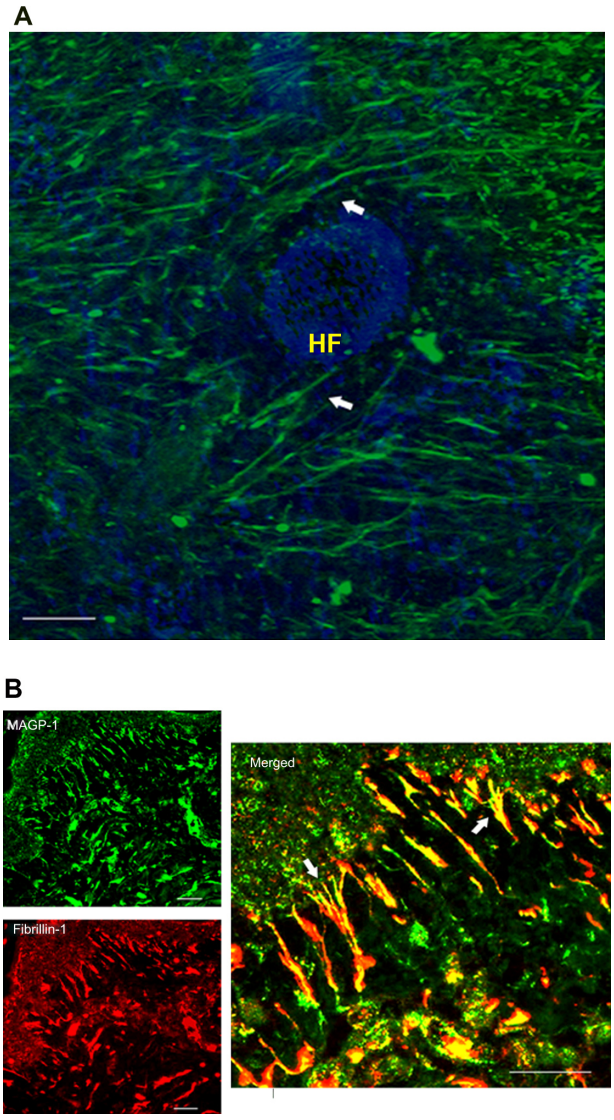

Figure 2 MAGP-1 protein localization in young skin.
Notes: (**A**) MAGP-1 expression in skin biopsy tissue. Representative image of confocal microscopy of a horizontal skin dermal section reveals that MAGP-1 is abundantly expressed in the dermis, and it forms the fibril structure. Green fluorescence represents MAGP-1, and the nuclei are visualized by DAPI stain. A HF is observed in the center of the section, and MAGP-1 wraps around the follicle, anchoring the follicle to surrounding the dermis (arrows). (**B**) Colocalization of MAGP-1 and fibrillin-1 in the dermis of human skin. MAGP-1 and a major extracellular matrix microfibril protein, fibrillin-1, were visualized in human skin biopsies using immunofluorescent staining (green, MAGP-1; red, fibrillin-1). These two proteins were found partially colocalized, and the majority of overlapping staining was observed on the oxytalan fibers at the papillary dermis underneath the dermal–epidermal junction (yellow stain, arrows). Scale bar: 50 μm.
Abbreviations: HF, hair follicle; MAGP-1, microfibril-associated glycoprotein-1; DAPI, 4′,6-diamidino-2-phenylindole.

MAGP-1 gene expression declines with age in human skin biopsies

Gene array from young and aged donors showed that the *MAGP-1* gene declined significantly (over twofold) in aged skin, along with a panel of elastic fiber-related genes (Table 1). The details of each donor are listed in Table 2. Although the

Table 2 Microfibril-associated glycoprotein-1 expression levels in all donors

Donors	Age	Group	MFAP-2 (MAGP-1) expression signal	Average	Standard error	*P*-value
1	34	Control	2,144.48	1,722.83	232.46	0.023
2	38	Control	1,681.65			
3	43	Control	1,342.36			
4	73	Experimental	641.96	815.81	98.61	
5	76	Experimental	822.11			
6	78	Experimental	983.37			

Abbreviations: MFAP-2, microfibrillar-associated protein; MAGP-1, microfibril-associated glycoprotein-1.

elastin gene only showed a trend of age-related decline (not significant), critical microfibril components (such as fibrillin-1 and MAGP-1), as well as "tethering" proteoglycans (such as biglycan and decorin), and elastic fiber and cell interface molecules (such as fibulin-5) had significantly lower levels of expression in aged skin. This change may contribute to atrophy, elasticity loss, and fragility in chronically aged skin.

UV irradiation disrupted the MAGP-1 protein network in vitro

Unlike intrinsic aging that is preprogrammed genetically, extrinsic aging primarily results from exposure to UV light and other environmental insults. It has been previously reported that nearly 80% of facial aging can be attributed to UV exposure.[17] Changes in the functionality of the dermal extracellular components account for the major visible changes that are associated with UV-induced skin damage.[2,3] Therefore, we evaluated the impact of UV irradiation on MAGP-1 protein in vitro and in vivo. In dermal fibroblast cultures, it was observed that MAGP-1 forms very fine and relatively short fibrils compared to fibrillin-1 (Figure 3A; left panels, arrows). After repetitive low-dose UV exposure, a significant reduction in the MAGP-1 level was observed (Figure 3A; upper right). This protein lost its regular fibril staining pattern and became sparse and disoriented, suggesting that UV inhibits the formation and/or accelerates the structural degradation of the MAGP-1 protein network. Similar changes were observed for fibrillin-1 (Figure 3A; lower right). The level of protein change with UV irradiation, as described, was quantified by Western blot analysis. Experiments were repeated three times. A significant reduction in the MAGP-1 protein level was observed, as quantified by ImageJ ($P<0.05$) (Figure 3B).

MAGP-1 declines in dermis with chronological aging, and photoexposure accelerates this process

Next, we observed the in vivo change of MAGP-1 in human skin. Full thickness biopsies from photoprotected and

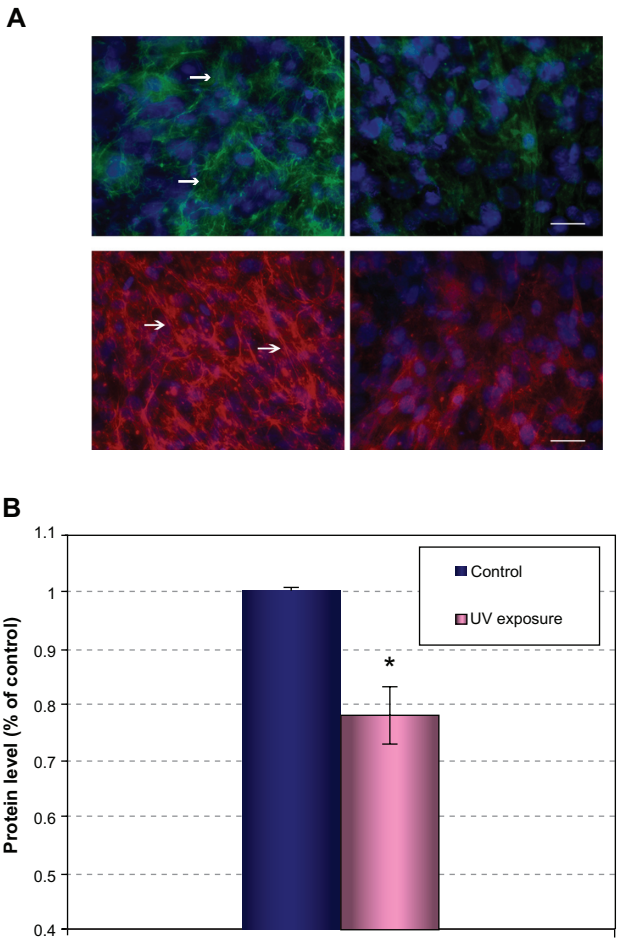

Figure 3 Reduction of MAGP-1 and FBN-1 levels after UV irradiation in dermal fibroblast culture.

Notes: (**A**) MAGP-1 forms very fine and relatively short fibrils (upper left; green fluorescence). After repetitive low-dose UV exposure, a significant reduction in the MAGP-1 level was observed (upper right). Cell nuclei were double-stained by DAPI as a control of cell number. FBN-1 forms relatively thicker and longer fibrils in culture (lower left; red fluorescence), as compared to MAGP-1. Similar changes were observed for FBN-1 after repetitive UV exposure (lower right). Immune reactivity seemed to be more confined within the cell body; it did not appear to exhibit an extracellular pattern (lower right; arrows). Cell nuclei were double-stained by DAPI as a control of cell number. Scale bar, 20 μm. (**B**) Western blot analysis showed the quantity of protein reduction after UV exposure. The intensity of each protein band was calculated by ImageJ (National Institutes of Health, Bethesda, MD, USA). Protein loading quantity is normalized by GAPDH control. The result shown is the average of three experiments, and the *P*-value was calculated by Student's *t*-test. *Indicates $P<0.05$.

Abbreviations: UV, ultraviolet; MAGP-1, microfibril-associated glycoprotein-1; FBN-1, fibrillin-1; DAPI, 4′,6-diamidino-2-phenylindole; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

photoexposed areas were examined to understand the in vivo distribution and expression pattern of this protein (Figure 4). In photoprotected skin (similar to the gene array data), a significant decline in the MAGP-1 protein was observed in aged skin (from the 57–79-year-old group) compared to the young skin (from the 18–21-year-old group). Such change was not apparent in the middle-aged group (34–42 year-olds) (Figure 4A). However, in photoexposed skin, a change in the MAGP-1 staining pattern was observed early in life. Around the late 30s, the MAGP-1 fibers became more "wavy" and fragmented, and the candelabra-like structures in the papillary dermis were significantly reduced compared to the younger group (Figure 4B; arrows). In the aged group, a large amount of amorphous solar elastosis-like material accumulated in the dermis (Figure 4B; arrowheads). This phenomenon can be observed from donors in their late 50s. As age progresses, a near complete loss of MAGP-1 immunoreactivity was observed (Figure 4; 79 year-olds). Similar age-related changes were observed in fibrillin-1 immunostaining, supporting the findings by Watson et al.[18]

Significant changes in skin structure and elasticity profile are often reported in women of postmenopausal age.[1,19] However, as Uitto[3] pointed out, such changes can be accelerated greatly by sun exposure. Our study demonstrated that the disruption of the underlying microfibril foundation of the skin can happen as early as in the 30s in photoexposed skin. This suggests that possible damage of the MAGP-1 fibrils with chronic photoaging can happen relatively early in life.

Progression of MAGP-1 loss in reticular dermis and around hair follicles with photoaging

The reticular dermis, rich in mature elastic and collagen fibers, provides density and strength to the skin, and also anchors the hair follicles. A cumulative effect of photoexposure can lead to the degradation of elastin and fibrillin in the reticular dermis.[18,20] In the reticular dermis of young skin, an abundant amount of MAGP-1 fibers, oriented parallel to the skin's surface, was observed. These fibril structures were also found to be wrapping around the hair follicles (Figure 4A; arrowheads), suggesting that these fibrils may play a role in providing structural support to anchor the fibers and to regulate the elasticity of follicles and pores. In the aged group, a significant reduction in the MAGP-1 level in both the reticular dermis as well as in areas surrounding the hair follicles was observed (Figure 4B; arrowheads). This suggests that declines in MAGP-1 may contribute to the disruption in the structural integrity of follicular regions in aged skin.

Discussion

MAGP-1 has been reported as a critical component of elastic fiber, and it plays an important role in maintaining the structure of elastic tissue.[1–3] However, its role during

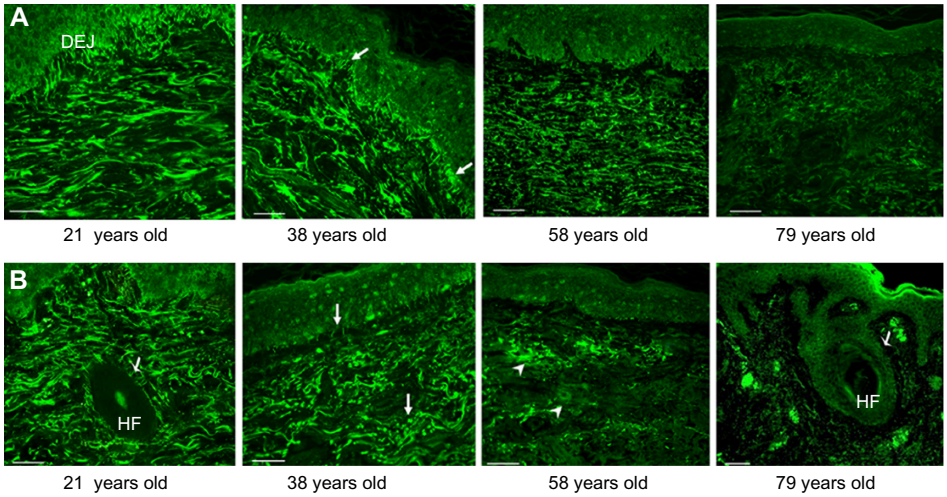

Figure 4 Age-related change of the MAGP-1 protein in human skin biopsies.
Notes: (**A**) MAGP-1 protein level declines in chronologically aged, photoprotected skin. MAGP-1 staining from biopsy samples of the upper underarms or thighs was demonstrated here. Young skin (18–21 years old; n=13) and middle-aged skin (34–42 years old; n=10) showed similar staining patterns and intensity in photoprotected area. A significant decline in staining was observed in the aged group (57–79 years old; n=12). (**B**) Photoexposure accelerates MAGP-1 loss in papillary and reticular dermis, as well as in the follicular regions. MAGP-1 fibers became more "wavy" and fragmented, and the candelabra-like structures in the papillary dermis were significantly reduced compared to the younger group (arrows). In the aged group, a large amount of amorphous solar elastosis-like material accumulates in the dermis (arrowheads). This phenomenon is very apparent from donors who are in their late 50s and older. As photoaging progresses, a near complete loss of MAGP-1 immunoreactivity was observed; also noted was a significant reduction of MAGP-1 levels in both the reticular dermis as well as in the areas surrounding the hair follicles (arrowheads). Scale bar: 50 μm.
Abbreviations: MAGP-1, microfibril-associated glycoprotein-1; n, number; DEJ, dermal-epidermal junction; HF, hair follicle.

human skin aging was not previously investigated. This study demonstrated that MAGP-1 colocalized with key microfibril components in the papillary dermis and dermal vasculatures, and that UV irradiation can damage this protein network. Furthermore, a decline in MAGP-1 expression with age was observed both at the gene level and at the protein level. Such change was accelerated by photoexposure and can be observed as early as in one's 30s, which represents a relatively young age group. Interestingly, age-related changes in other organs and systems can be observed at a similar age, as described by Ford,[21,22] suggesting that systematic alterations in the human body's functions begins to manifest in this age group. In the skin (and in addition to UV damage), cumulative impacts from combined internal and external aging factors such as gene modulation, hormone changes, oxidative stress, climate and dryness, lifestyle, and so on, could all play a part in the skin's aging process.

Long-term photoaging appears to result in the progression of MAGP-1 loss in the follicular regions and in the reticular dermis, which may lead to fragility and instability of the dermal matrix, and contribute to the various signs of an aged appearance such as wrinkles, sagging, loss of elasticity and bounciness, and enlarged pores.

While collagen comprises a major part of the protein network that contributes to the firmness and tensile strength of skin, the healthy elastic fibers are important for the stretching and recoiling of the tissue, and they are also critical in tissue integrity as well. The microfibril components associated with elastin help regulate the formation, assembly, and stabilization of elastic fibers, and they are also critical for dermal integrity and elasticity. Such components should not be overlooked in antiaging investigations. An interesting observation in this study is that the dermal MAGP-1 level can significantly decline under the dermal–epidermal junction at a relatively young age, when the majority of collagen and elastic fibers have been reported to still remain intact.[23] Therefore, methods for the early prevention or correction of such changes should be adopted to avoid further diminishment and subsequent damage to the dermal foundation. Further functional assays pertaining to MAGP-1, such as gene knock-down studies in skin cells and tissues, should be carried out to better elucidate how this type of age-related change affects the total elastic network.

In summary, our studies helped to better understand the role of MAGP-1 during skin aging, and the findings indicated that the loss of MAGP-1 might contribute to or accelerate the visible signs of aging in the skin. The modulation of MAGP-1 or the prevention of protein loss may provide a new way to help reestablish elastic properties in the skin.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Calleja-Agius J, Muscat-Baron Y, Brincat MP. Skin ageing. *Menopause Int*. 2007;13(2):60–64.
2. Jenkins G. Molecular mechanisms of skin ageing. *Mech Ageing Dev*. 2002;123(7):801–810.
3. Uitto J. The role of elastin and collagen in cutaneous aging: intrinsic aging versus photoexposure. *J Drugs Dermatol*. 2008;7(Suppl 2): s12–s16.
4. Segade F. Functional evolution of the microfibril-associated glycoproteins. *Gene*. 2009;439(1–2):43–54.
5. Tsuruga E, Yajima T, Irie K. Microfibril-associated glycoprotein-1 and fibrillin-2 are associated with tropoelastin deposition in vitro. *Int J Biochem Cell Biol*. 2005;37(1):120–129.
6. Craft CS, Broekelmann TJ, Zou W, Chappel JC, Teitelbaum SL, Mecham RP. Oophorectomy-induced bone loss is attenuated in MAGP1-deficient mice. *J Cell Biochem*. 2012;113(1):93–99.
7. Hayes AJ, Smith SM, Gibson MA, Melrose J. Comparative immunolocalization of the elastin fiber-associated proteins fibrillin-1, LTBP-2, and MAGP-1 with components of the collagenous and proteoglycan matrix of the fetal human intervertebral disc. *Spine (Phila Pa 1976)*. 2011;36(21):E1365–E1372.
8. Chen E, Larson JD, Ekker SC. Functional analysis of zebrafish microfibril-associated glycoprotein-1 (Magp1) in vivo reveals roles for microfibrils in vascular development and function. *Blood*. 2006;107(11): 4364–4374.
9. Werneck CC, Vicente CP, Weinberg JS, et al. Mice lacking the extracellular matrix protein MAGP1 display delayed thrombotic occlusion following vessel injury. *Blood*. 2008;111(8):4137–4144.
10. Baumann L. Skin ageing and its treatment. *J Pathol*. 2007;211(2): 241–251.
11. Wagenseil JE, Mecham RP. New insights into elastic fiber assembly. *Birth Defects Res C Embryo Today*. 2007;81(4):229–240.
12. Reinboth B, Hanssen E, Cleary EG, Gibson MA. Molecular interactions of biglycan and decorin with elastic fiber components: biglycan forms a ternary complex with tropoelastin and microfibril-associated glycoprotein 1. *J Biol Chem*. 2002;277(6):3950–3957.
13. Weinbaum JS, Tranquillo RT, Mecham RP. The matrix-binding domain of microfibril-associated glycoprotein-1 targets active connective tissue growth factor to a fibroblast-produced extracellular matrix. *Macromol Biosci*. 2010;10(11):1338–1344.
14. Clarke AW, Weiss AS. Microfibril-associated glycoprotein-1 binding to tropoelastin: multiple binding sites and the role of divalent cations. *Eur J Biochem*. 2004;271(14):3085–3090.
15. Jensen SA, Reinhardt DP, Gibson MA, Weiss AS. Protein interaction studies of MAGP-1 with tropoelastin and fibrillin-1. *J Biol Chem*. 2001;276(43):39661–39666.
16. Trask BC, Trask TM, Broekelmann T, Mecham RP. The microfibrillar proteins MAGP-1 and fibrillin-1 form a ternary complex with the chondroitin sulfate proteoglycan decorin. *Mol Biol Cell*. 2000;11(5): 1499–1507.

17. Uitto J. Understanding premature skin aging. *N Engl J Med*. 1997;337(20):1463–1465.
18. Watson RE, Griffiths CE, Craven NM, Shuttleworth CA, Kielty CM. Fibrillin-rich microfibrils are reduced in photoaged skin. Distribution at the dermal-epidermal junction. *J Invest Dermatol*. 1999;112(5):782–787.
19. Bolognia JL, Braverman IM, Rousseau ME, Sarrel PM. Skin changes in menopause. *Maturitas*. 1989;11(4):295–304.
20. Watson RE, Ball SG, Craven NM, et al. Distribution and expression of type VI collagen in photoaged skin. *Br J Dermatol*. 2001;144(4):751–759.
21. Ford JH. Protraction of anaphase B in lymphocyte mitosis with ageing: possible contribution to age-related cancer risk. *Mutagenesis*. 2013;28(3):307–314.
22. Ford JH. Saturated fatty acid metabolism is key link between cell division, cancer, and senescence in cellular and whole organism aging. *Age (Dordr)*. 2010;32(2):231–237.
23. El-Domyati M, Attia S, Saleh F, et al. Intrinsic aging vs photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Exp Dermatol*. 2002;11(5):398–405.