Matrix Biology 33 (2014) 41–46

Contents lists available at ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

Brief report

Differential expression of cathepsins K, S and V between young and aged Caucasian women skin epidermis

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ARTICLE INFO

Article history:
Received 27 May 2013
Received in revised form 8 July 2013
Accepted 9 July 2013

Keywords:
Aging
Cysteine proteases
Cystatins
Keratinocytes
Nidogen-1

ABSTRACT

Cutaneous aging translates drastic structural and functional alterations in the extracellular matrix (ECM). Multiple mechanisms are involved, including changes in protease levels. We investigated the age-related protein expression and activity of cysteine cathepsins and the expression of two endogenous protein inhibitors in young and aged Caucasian women skin epidermis. Immunofluorescence studies indicate that the expression of cathepsins K, S and V, as well as cystatins A and M/E within keratinocytes is reduced in photoprotected skin of aged women. Furthermore, the overall endopeptidase activity of cysteine cathepsins in epidermis lysates decreased with age. Albeit dermal elastic fiber and laminin expression is reduced in aged skin, staining of nidogen-1, a key protein in BM assembly that is sensitive to proteolysis by cysteine, metallo- and serine proteases, has a similar pattern in both young and aged skin. Since cathepsins contribute to the hydrolysis and turnover of ECM/basement membrane components, the abnormal protein degradation and deposition during aging process may be related in part to a decline of lysosomal/endosomal cathepsin K, S and V activity.

1. Introduction

Skin aging is the result of two biological processes (i.e. chronological or intrinsic aging and photoaging due to UV exposure) that both share important molecular features including drastic structural and functional alterations particularly in the extracellular matrix (ECM) and dermal–epidermal junction (DEJ) (Rittié and Fisher, 2002). The hallmark of clinical aged skin manifestations is characterized by wrinkle formation in the epidermis, sagging, thinning, dryness, a reduced immune response and a slow wound healing (Robert et al., 2009). It is believed that multiple mechanisms are involved, including changes in the expression of proteases. Indeed, at the molecular level, aged skin is accompanied by the accumulation of reactive oxygen species (ROS) that up-regulate various matrix metalloprotease (MMP) production, leading to an increased degradation of ECM and accumulation of non-functional matrix components. Besides MMPs (e.g. MMP-1, -3 and -9) and serine proteases (e.g. elastase and cathepsin G), which have been extensively investigated in aged skin (Pillai et al., 2005), few reports related to cysteine cathepsins were mainly focused on their expression and roles in dermal fibroblasts during photoaging or skin inflammation events (Codriansky et al., 2009; Klose et al., 2010; Lai et al., 2010; Zheng et al., 2011). These proteolytic enzymes (11 members belonging to clan CA family C1), have long been regarded as lysosomal house-keeping enzymes which ensure the degradation and the recycling of endocytosed proteins. Cysteine cathepsins also participate in specific cellular proteolytic processes in human skin, such as hair follicle morphogenesis, basement membrane (BM)/ECM turnover, skin color, apoptosis and senescence (Tobin et al., 2002; Büth et al., 2004; Bivik et al., 2006; Chen et al., 2006; Rünger et al., 2007; Kraus et al., 2011; Ebanks et al., 2013) as well as various pathophysiological events (e.g. tumorigenesis, metastasis, inflammation, psoriasis, Papillon–Lefevre syndrome) (Lecaille et al., 2002; Turk et al., 2012). Furthermore, cysteine cathepsins as well as their cognate inhibitors, the cystatins A and M/E, play an important role in epidermal differentiation (for review: (Brocklehurst and Philpott, 2013)). Nevertheless, the status of cysteine cathepsins and their endogenous inhibitors in epidermis in elderly people is still lacking. In the present study, we investigated for the first time the age-related protein expression and activity of cysteine cathepsins in young and aged Caucasian women skin epidermis by immunofluorescence, Western-blot and kinetics studies. Furthermore, we evaluated by immunofluorescence the protein expression of cystatins A and M/E and two basement membrane constituents from the dermal–epidermal junction (laminin-332/-511 and nidogen-1) that are both potential substrates of cysteine cathepsins.

Abbreviations: AMC, 7 amino-4 methylcoumarin; DEJ, dermal–epidermal junction; DTT, dithiothreitol; ECM, extracellular matrix; E-64, L-3-carboxy-trans-2, 3-epoxypropionyl-leucylamido-(4-guanidino) butane; MMP, matrix metalloprotease; Z, Benzyloxycarbonyl.

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http://dx.doi.org/10.1016/j.matbio.2013.07.002

2. Results and discussion

Cryosections of photoprotected young and aged human epidermis were immunostained with anti-human cathepsins B, K, L, S or V antibodies, respectively (Fig. 1A). Cathepsins B, K, L, S and V are detected broadly within keratinocytes (most likely in the endosomal/lysosomal compartment) throughout all epidermal layers of young and aged donors. Despite that the epidermal thickness in photoprotected young (44.8 ± 5.2 μm) and aged (42.15 ± 6.0 μm) donors remained constant as described elsewhere (El-Domyati et al., 2002), staining of cathepsins

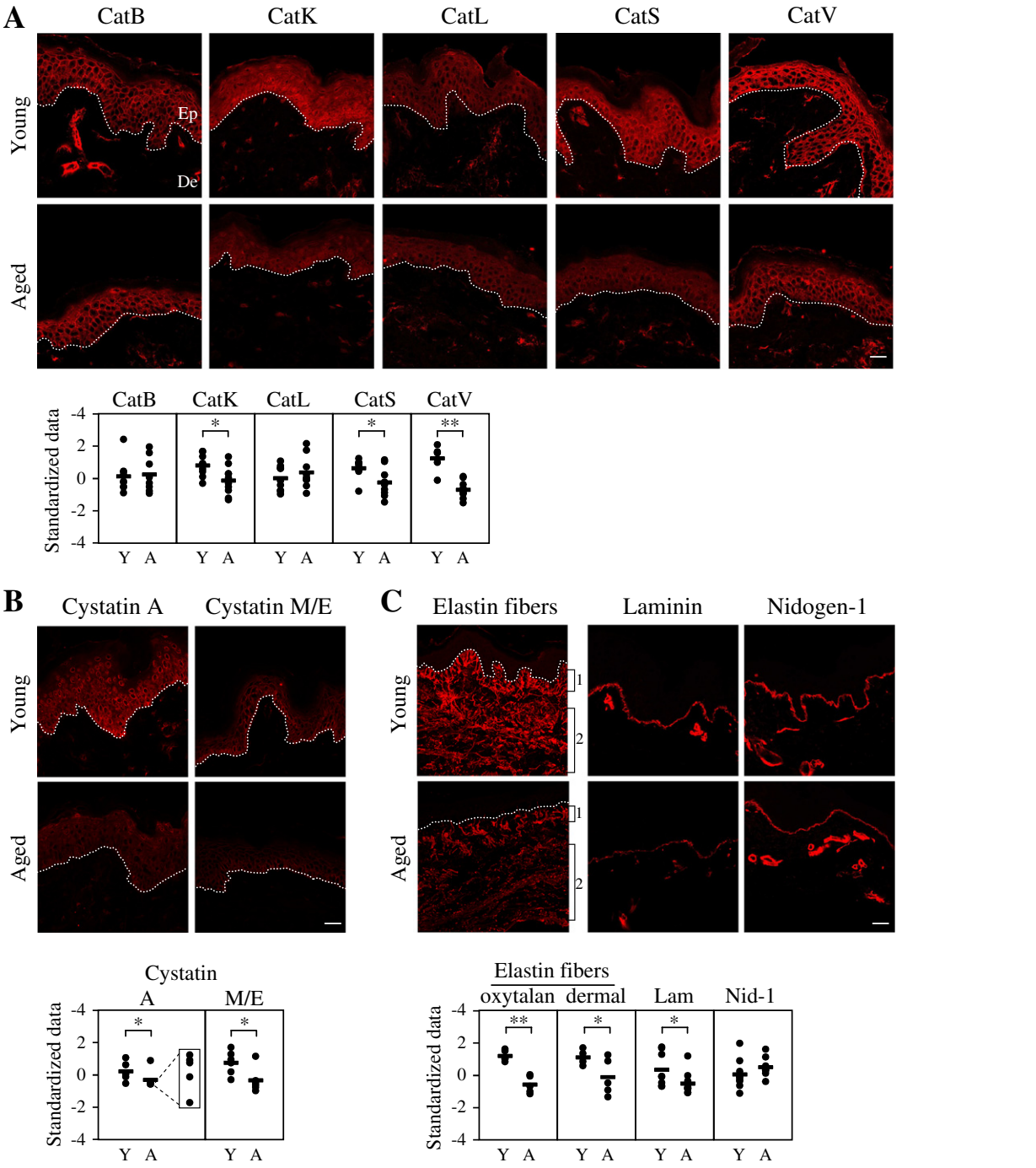

Fig. 1. Age-related changes in cathepsins, cystatins and ECM proteins in human skin. A) Immunofluorescence staining of cathepsins B, K, L, S and V in epidermis (photoprotected arm), using specific polyclonal antibodies that recognize both pro- and mature forms to each cathepsins (Sage et al., 2012). Representative pictures of skin biopsies of young (19–27 years; average age: 21.6 ± 2.5 years) and aged (61–68 years; average age: 65.7 ± 3.2 years) donors are shown. Following confocal laser microscopy acquisition, pictures were analyzed and relative expression of cathepsins was quantified and standardized to the epidermis surface (Leica QWin software). B) Immunofluorescence staining of cystatins A and M/E. Expression levels of cystatins A and M/E were quantified and standardized to the epidermis surface. C) Immunofluorescence staining of elastin fibers, laminins and nidogen-1. Oxytalan and dermal fibers are located in zones 1 and 2, respectively. Relative expression levels of ECM proteins (elastin fibers) in the dermis and BM components (laminin-332/-511 and nidogen-1) in the DEJ surface. Respective oxytalan and dermal fiber expression was standardized to the upper dermis surface near the DEJ and the deeper dermis surface. Quantification of basement membrane protein expression was standardized to the dermal–epidermal junction surface. Data are shown as individual points and horizontal bars indicate means. Statistical significance between young and aged donors was assessed using two-way ANOVA (statistically significant differences are denoted with asterisks: *, $P \leq 0.05$; **, $P \leq 0.01$). Scale bar = 25 μm. Ep: epidermis, De: dermis, Y: young, A: aged. Lam: laminin, Nid-1: nidogen-1. The dashed line indicates the basement membrane.