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Original Contribution

AGE-DEPENDENT INCREASE OF COLLAGENASE EXPRESSION CAN BE REDUCED BY α-TOCOPHEROL VIA PROTEIN KINASE C INHIBITION

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Abstract—Total protein kinase C (PKC) activity in human skin fibroblasts increases during in vivo aging as a function of the donor's age. During in vitro aging protein kinase C activity is also increased, as a function of cell passage number. Using PKC isoform specific antibodies, we demonstrate that the increase in total PKC activity is mainly due to the PKC α isoform. PKC α protein expression increased up to 8 fold during in vivo aging. Collagenase (MMP-1) gene transcription and protein expression also increased with age, concomitant with the increase in protein kinase C α. Furthermore, α-tocopherol, which inhibits protein kinase C activity, is able to diminish collagenase gene transcription without altering the level of its natural inhibitor, tissue inhibitor of metalloproteinase, TIMP-1. We propose that an aging program leads to increased protein kinase C α expression and activity. This event would induce collagenase overexpression followed by increased collagen degradation. Our in vitro experiments with skin fibroblasts suggest that α-tocopherol may protect against skin aging by decreasing the level of collagenase expression, which is induced by environmental insults and by aging. © 1999 Elsevier Science Inc.

Keywords—Collagenase, PKC, Tocopherol, Aging, Fibroblasts, Free radicals

INTRODUCTION

The diminution of the extracellular matrix is a common event during the aging of connective tissues. Human skin fibroblasts from older donors have increased levels of collagenase mRNA and protein, relative to younger donors [1], whereas expression of the collagen type I and III genes decrease in an age dependent way [2,3]. Replicative senescence of human skin fibroblasts appears to correlate with a loss of regulation and overexpression of collagenase activity [4].

In humans, three collagenases have been described: interstitial collagenase-1 (MMP-1), present in many cell types; neutrophil collagenase (MMP-8) and the recently discovered collagenase-3 (MMP-13), which is mainly present in chondrocytes [5–9]. Transcription of the human collagenase-1 gene (MMP-1), the main collagenase in skin fibroblasts, is induced by phorbol esters and repressed by glucocorticoids [1,10,11]. Both types of regulation are mediated by an AP1 element located between positions −73 and −65 in the promoter sequence [10]. The AP1 transcription factor family is induced by phorbol esters and regulates expression of collagenase in response to various extracellular signals [12,13]. Protein kinase C (classical and novel isozymes) is primarily responsible for the transcriptional regulation of phorbol ester-inducible genes [14,15]. Collagen-stimulated induction of collagenase was found to be mediated by tyrosine kinase and protein kinase C activities [16].

The studies discussed above show that aging is associated with an increased collagenase expression, but the age dependent regulatory mechanisms involved in the overexpression of collagenase have not yet been elucidated. Expression of collagenase is under the control of PKC via the AP1 phorbol ester responsive element [11–13], and age dependent changes in this pathway could possibly lead to an overall increase in collagenase expression.

In the present study, we have investigated the mech-

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anism of age dependent increase in collagenase (MMP-1) expression and whether this event is counteracted by an increase of its natural inhibitor, tissue inhibitor of metalloproteinase 1, TIMP-1. This study shows that the increased expression of collagenase (MMP-1) is correlated with an age dependent increase of PKC activity, which can be mainly attributed to an increase of PKC α expression. It shows also that the age dependent collagenase (MMP-1) expression can be diminished by α-tocopherol, consistent with the observation that α-tocopherol is able to decrease PKC activity in a number of cells, as well as in fibroblasts [17,18]. These findings, if extrapolated to a clinical level, may suggest a role for α-tocopherol as a natural skin aging-prevention agent.

MATERIALS AND METHODS

Materials

Tissue culture materials were purchased from Falcon Labware (Becton Dickinson AG, Basel) and Gibco Laboratories (Grand Island, NY, USA). Growth media, serum for cell culture, and anti-PKC isozyme-specific antibodies were obtained from Gibco Laboratories. A mouse monoclonal antibody raised against the oligopeptide corresponding to residues 332 to 350 of human collagenase (MMP-1) was purchased from Calbiochem. Human female skin fibroblasts (ATCC#: CCD-39SK (1 week old, passage 5), CCD-919SK (19 years old, passage 2), CCD-1074SK (42-years old, passage 1), CCD-974SK (61-years old, passage 2) and CCD-1069SK (70-years old, passage 1)), human MMP-1 cDNA (P35-1, ATCC# 79063) and TIMP-1 genomic DNA (TIMP-3.9, ATCC# 59666) were purchased from the American Type Culture Collection (Rockville, MD, USA). Phorbol 12-myristate 13-acetate (PMA) and streptolysin–O (25,000 units) were from Sigma (Buchs, Switzerland). The enhanced chemiluminescence western blotting analysis system was purchased from Amersham International. α-Tocopherol was a generous gift from Henkel Corporation (LaGrange, USA). A peptide derived form of bovine myelin basic protein (MBP_{4-14}) (residues 4–14) was used as PKC phosphatase resistant substrate for total PKC activity assays (Bachem, Dubendorf, Switzerland). All other chemicals used were of the purest grade commercially available.

Cell culture

Human skin fibroblasts were grown in Dulbecco's modified Eagle medium (DMEM) containing 25 mM sodium bicarbonate, 60 U/ml penicillin, 60 μg/ml streptomycin and 10% fetal calf serum (FCS). Cells were usually seeded into 75 cm^2 flasks and grown to confluence at 37°C in a humidified atmosphere of 5% CO_2. Media and sera used in all experiments were from the same batch number and source. Cells between passage 3 and 22 were used for all the experiments. Experiments were repeated with cells at consecutive passages as indicated in the figure legends. Viability was determined by the trypan blue exclusion method. Cells in a subconfluent state were made quiescent by incubating them in media containing 0.2% FCS for at least 48 h. α-Tocopherol was added to the cultures as ethanol solution or adsorbed to FCS as described [19] at the indicated concentrations.

Determination of PKC activity in permeabilized cells

Activity of PKC in permeabilized fibroblasts was performed according to the procedure of Alexander et al. [20] with minor modifications. Quiescent cells were subjected to different treatments as indicated. During the last hour of the preincubation period, cells were treated with 100 nM phorbol 12-myristate 13-acetate (PMA). Then, cells were washed twice with PBS, resuspended in intracellular buffer (5.2 mM $MgCl_2$, 94 mM KCl, 12.5 mM N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), 12.5 mM egtazic acid (EGTA), 8.2 mM CaCl, pH 7.4) and divided in 220 μl portions (1.5×10^5 cells). Assays were started by adding [γ-^{32}P]ATP (9 cpm/pmol, final concentration 250 μM), peptide substrate (final concentration 70 μM), and streptolysin-O (0.3 IU). The reaction mixtures were incubated at 30°C for 10 min and the reaction was stopped by addition of 100 μl of 25% (w/v) trichloroacetic acid in 2 M acetic acid. After 10 min on ice, 100 μl aliquots were spotted onto 4.5 cm $\times$ 4.5 cm P81 ion exchange chromatographic paper within predrawn squares (Whatman International). The paper was then washed twice for 10 min. with 30% (v/v) acetic acid containing 1% phosphoric acid and once with ethanol. The amount of the two washes was 10 ml each per spotted square. The P81 paper was dried, the squares cut and radioactivity was counted in a liquid scintillation analyser. Basal phosphorylation in the absence of the peptide was subtracted from the experimental data to determine the specific activity.

Western blot analysis

Cells were harvested by trypsinisation and resuspended in a small volume of ice cold disruption buffer (Tris-HCl 20 mM, ethylenediaminetetraacetate (EDTA) 5 mM, EGTA 2 mM, pH 7.4) containing protease inhibitors. Following disruption by sonication (10 bursts at 20 W), samples were centrifuged (10,000 $\times$ g, 15 min 4°C) and supernatants stored at -70°C until analysis. Proteins (5 μg per lane) were separated using a 10% SDS-PAGE

separating gel [21] and electro-blotted onto Immobilon-P (Millipore, Bedford, MA, USA) membrane. Each PKC isoenzyme was detected using the corresponding antibody and visualized by chemiluminescence (ECL, Amersham, Braunschewig, Germany) following labeling with horseradish peroxidase-labeled anti-rabbit IgG diluted at 1:10,000 (Amersham). Rabbit anti-mouse actin antibody was used as primary antibody to control the amount of loaded protein, in addition to BCA protein assay (Pierce).

Preparation of the MMP-1, TIMP-1 and GAPDH probes

The plasmid P35-1 containing the human MMP-1 cDNA was cut with XhoI and a 2.2 kb cDNA fragment containing the complete coding sequence of collagenase (MMP-1) was gel purified. The plasmid containing a 3.9 kb human TIMP-1 genomic fragment (TIMP-3.9) was digested with BglI, separated with an agarose gel and a 694 bp fragment was gel-purified. Analysis of the partial sequence of the human TIMP-1 gene (Genbank accession number D11139) indicated that the 694 BglI fragment contained the entire exon 5 (126 bp) of the TIMP-1 gene and part of intron 4 and 5. The preparation of the fragment containing the coding region of rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as loading control has been described [22].

Northern blot analysis

Equal amounts of total RNA (10 μg) were size-fractionated on 1% (w/v) agarose gel in 20 mM MOPS, 5 mM Na-acetate, 0.5 mM EDTA, pH 7.0 containing 2 M (6%) formaldehyde. RNA was transferred to a Zeta-Probe membrane (Bio-Rad Laboratories, Hercules, CA, USA) using 10 × SSC (SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and cross-linked to the membrane using UV irradiation. The probes were labeled with [α-^{32}P]dATP using the random prime labeling kit (Boehringer-Mannheim, Mannheim, Germany). Unincorporated label was removed using NICK spin columns (Pharmacia Biotech, Piscataway, NJ, USA). Prehybridization (30 min) and hybridization (overnight) was performed at 42°C in 50% formamide, 0.25 M NaCl, 0.12 M NaH_2PO_4, pH 7.2, 1 mM EDTA, 7% sodium dodecyl sulfate (SDS) and 2 × 10^6 cpm/ml of heat denatured [α-^{32}P]dATP-labeled cDNA probe. Blots were washed in 1 × SSC, 0.1% SDS at 25°C, then in 0.25 × SSC, 0.1% SDS at 50°C for 30 min. Blots were exposed to a Phosphorimager (Biorad) and the signals were quantified using the image quant program.

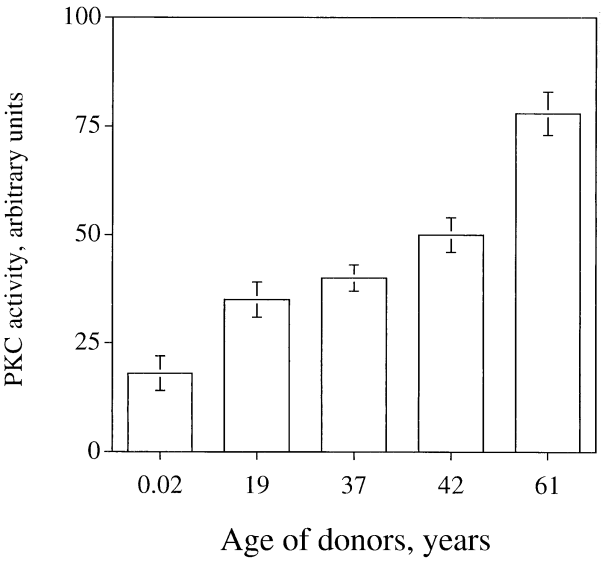

Fig. 1. Effect of aging on PKC activity in human skin fibroblasts. PKC activity was assayed in permeabilized cells as described in Materials and Methods. The results shown are the mean ± SD of five independent determinations. Cells at the same passage number were used (passage 10 to 14).

RESULTS

Total PKC activity in fibroblasts increases with age

Human skin fibroblasts from different donor age were grown in culture to sub-confluence and treated with 0.1 μM phorbol myristate acetate (PMA) for 1 hour. After cell permeabilisation with streptolysin-O, the activity of total PKC was measured with a peptide substrate and radioactive [γ-^{32}P]ATP. The activity measured under these conditions is always maximal and, therefore, cannot be attributed to an increase in the concentration of activators such as calcium, fatty acids or diacylglycerol. Figure 1 shows that the total maximal activity of PKC in human skin fibroblasts increases with age, at 61 years being four times higher than at 1 week.

The increase of total PKC activity during aging is due to PKC α

It is well known that protein kinase C belongs to a family of at least 12 isoforms, that are differently present in different cells. To determine which PKC isoform is responsible for the age dependent increase of total PKC activity, Western blot analysis using PKC isoform specific antibodies were carried out with total fibroblast extracts from donors of different age. We concentrated on the detection of α, δ, ϵ, and ζ isoforms, which are known to be present in fibroblasts (Fig. 2). The only isoform showing clear age dependent changes was

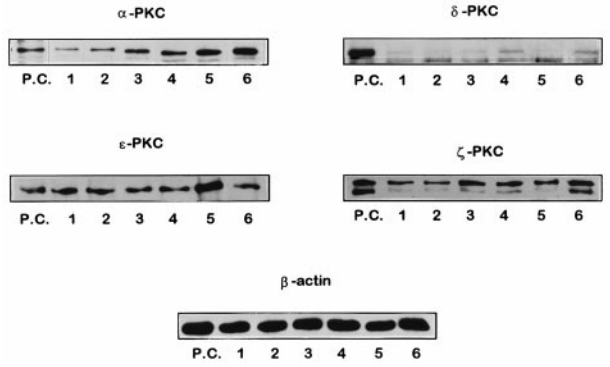

Fig. 2. Western blot analysis of PKC isoforms in human skin fibroblasts from different age-donors. Total cell extracts from cells at passage 10, 11 and 12 were prepared as described in Materials and Methods and analysed by SDS-PAGE followed by immunoblotting using PKC isoform specific antibodies. β-Actin was used as an internal control. The figure shows the result obtained with the cells at passage 10, which was confirmed with cells at passage 11 and 12 **1**: 1 week; **2**: 19 years; **3**: 37 years; **4**: 42 years; **5**: 61 years; **6**: 70 years; P.C.: positive control.

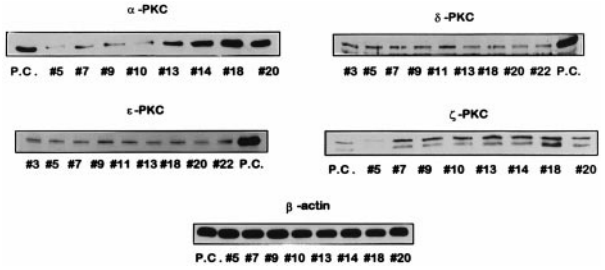

Fig. 3. Immunodetection of α, δ, ϵ, and ζ PKC isoforms in cells from a 19 years old donor at different passage numbers. Cell extracts were prepared as indicated in Materials and Methods. α-actin was used as internal control. The passage number indicates the real passage number (including the passage number given from ATCC). The figure shows one representative experiment performed in triplicate, with essentially similar results.

PKC α, which increased in the level of protein, while the levels of the other isoforms did not appear to be significantly affected by aging. PKC α expression was minimal at 1 week of age and increased up to eight-fold in the oldest donors. Although a weak increase can also be seen with PKC ζ, especially in the older donors, it was less significant. Thus, the increase in total PKC activity (Fig. 1) correlated well with the increase of PKC α protein. The molecular basis of the age-dependent expression of PKC α in fibroblasts is not known at the present time.

The PKC α isoform is upregulated during in vitro cell aging

In vitro replicative cell senescence has often been used as a model for the in vivo phenomenon of aging. We wondered whether different PKC isoforms were expressed differently during in vitro aging depending on the passage number of the cells in culture. Again, protein kinase C isoforms α, δ, ϵ, and ζ were studied by using isoform specific antibodies and Western blots (Fig. 3). Protein kinase C α expression increased with passage number of fibroblasts coming from a given donor (19-years old), whereas the other PKC isoforms tested remained unchanged. We conclude from the above data that PKC amount and activity in fibroblasts, both in an in vitro aging model as well as when assessed in cells from donors of different age, increase as a function of age.

Collagenase expression increases parallel to PKC α during aging

PKC is involved in transmitting signals from the extracellular medium to the nucleus, mainly via its ability to activate the transcription factor AP1. It was interesting to determine whether the age dependent increase of PKC α is paralleled by increased expression of enzymes that are under the control of PKC. Collagenase (MMP-1) is under the transcriptional control of a cis-activating element, TRE (TPA-responsive element) and it is likely that increases of PKC activity are a cause of higher collagenase expression during aging. Therefore, collagenase (MMP-1) levels in human skin fibroblasts from donors of different age were measured by immunoblotting using anti-collagenase antibodies (Fig. 4). Collagenase (MMP-1) protein expression was indeed increased as a function of donor age, in parallel to PKC activity.

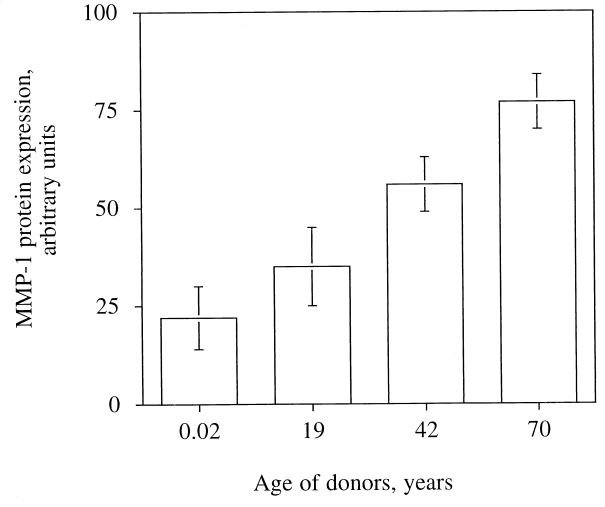

Fig. 4. Western blot analysis of MMP-1 in human skin fibroblasts from donors of different age. Total cell extracts were prepared as described in Materials and Methods section and analysed by SDS-PAGE followed by immunoblotting using a mouse monoclonal antibody raised against the oligopeptide corresponding to residues 332 to 350 of human MMP-1. The graph shows the result obtained with the cells at passage 10, 11, and 12.

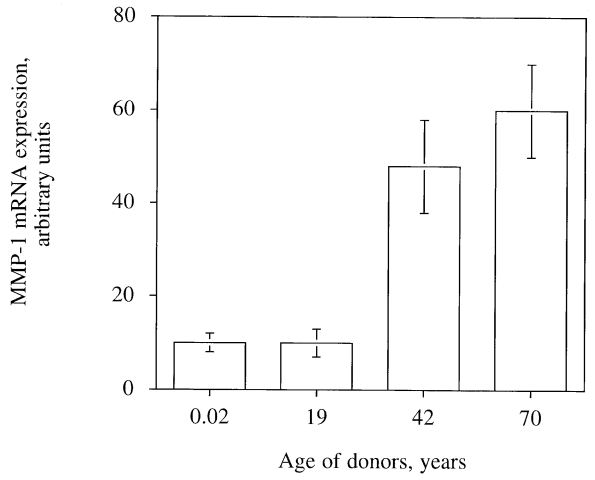

Fig. 5. Northern blot analysis of MMP-1 mRNA in human skin fibroblasts from donors of different age. Total RNAs were prepared as described in Material and Methods and probed with an MMP-1 cDNA. Samples were loaded in equal aliquots, based on UV light examination of ethidium bromide-stained gels. Final normalisation was obtained by hybridisation of the same Northern blot with a probe for GAPDH mRNA. Data are from experiments repeated three times with donor cells at the same passage number (from passage 12 to 14).

Transcription of the collagenase gene increases parallel to PKC α

To corroborate that the age dependent increase of collagenase (MMP-1) was due to increased expression of the MMP-1 gene and not to the decreased degradation of collagenase protein, Northern blots were carried out (Figure 5). MMP-1 mRNA levels in human skin fibroblasts increased as a function of the age of the fibroblast donors and paralleled the increase in PKC α−expression. The age-dependent increase of MMP-1 mRNA and protein were not identical, suggesting regulation at the level of translation or protein turnover. Interestingly, the higher expression of MMP-1 mRNA was not followed by an age dependent increase of TIMP-1 mRNA, which encodes the main collagenase-1 inhibitor (Table 1). Although both the MMP-1 and TIMP-1 genes are under the control of PKC and PMA, only the MMP-1 gene appears to be affected by the age-dependent increase of PKC α. This result suggests independent regulation of MMP-1 and TIMP-1 gene expression by PKC, as described already for these two genes in response to interleukin-1 [23], retinoic acid [24], TGF-β [23,25,26] and UV irradiation [27]. It is not possible, at this time, to estimate whether the absence of TIMP-1 mRNA upregulation provides a level of TIMP-1 protein able to inhibit the age-dependent collagenase increase. However, the experiments presented above support the conclusion that the increase of PKC α expression may be the consequence of an intrinsic cellular aging program which also affects events downstream of PKC. Consistent with this, we find an increased expression of the collagenase (MMP-1) mRNA which is under PKC control. These results clearly suggest a possible molecular mechanism for age-dependent collagen degradation.

Table 1. TIMP-1 mRNA Levels in Fibroblasts of Different Age as Measured With Northern Blots

Donor age (y)	Relative level[a]	Fold induction by[b]	
		PMA	PMA + α-tocopherol
0.02	3.8 ± 1.1	3.0 ± 0.7	2.6 ± 0.6
19	3.2 ± 0.8	2.8 ± 1.2	2.2 ± 0.9
42	5.9 ± 1.0	2.5 ± 1.1	2.1 ± 0.6
70	4.0 ± 1.1	3.1 ± 0.5	3.1 ± 0.7

[a] Relative levels of TIMP-1 mRNA normalised to GAPDH mRNA control. Data from two independent experiments performed with cells at passage 12 and 13.
[b] PMA was at 0.1 μM and α-tocopherol at 50 μM.

Collagenase expression is inhibited by α-tocopherol

The notion that PKC α activity can be modulated by α-tocopherol is a fundamental part of a number of studies carried out in our laboratory [17,18,28–30]. We have shown that PKC α is less active when cells are incubated with α-tocopherol [31]. This inhibition is specific for α-tocopherol and is not shared by other tocopherol isomers. We therefore assessed whether inhibition of PKC α by α-tocopherol would decrease the transcription of MMP-1 mRNA. The incubation of fibroblasts from different donor's age with α-tocopherol resulted in a substantial diminution of transcription of MMP-1 mRNA with all ages tested, except for one week (Fig. 6). The actual increase observed in the newborn might be relevant in connection with the plasticity and the tissue growth needs during the pre-puberty period. Thus, the age-dependent expression of collagenase may be modified by α-tocopherol at a concentration (50 μM) which is within the physiologic range [30].

DISCUSSION

In culture, human diploid fibroblasts show a well characterized limited division potential, which is both dependent on the age of the donor and on the number of population doublings in culture [32,33]. Various causes for cellular senescence in vitro and in vivo have been described: oxidative stress; telomere shortening; and the accumulation of replication errors all contribute to cellular senescence [34,35]. The limited division potential of cells is clearly not the only effect of cellular senescence, since the expression of a number of genes changes

Fig. 6. Northern blot analysis of MMP-1 mRNA in human skin fibroblasts from donors of different age after α-tocopherol treatment. The experiment was carried out as described in the legend to Fig. 5, except that 50 μM α-tocopherol was added. The data are calculated as percent of the α-tocopherol treated sample relative to the untreated control. Data are from experiments repeated two times with donor cells at the same passage number (from passage 15 to 16).

progressively both as a result of in vivo and in vitro aging. In fact, altered cellular physiology and changes in gene expression during aging could explain some of the symptoms and diseases associated with increased age, such as degeneration of the skin and increased susceptibility to tissue degeneration and inflammation. The activity of a number of enzymes such as catalase decreases during aging, whereas for other enzymes, such as glutathione peroxidase, it increases [36]. Enzymes such as ubiquitin carboxyl-terminal transferase and procollagen A_IIII are processed differently in an age-dependent way [37]. These changes occur well before cells reach their limited division potential and are the result of both genetic aging programs and environmental insults. At the molecular level, expression of genes such as collagenase and stromelysin [1,4,23] TIMP-2 [23,38] fibronectin [39], p21$^{Waf1/Cdi1/Ssi1}$ [40], and p16^{CDKN2} [41], is known to increase during aging, whereas for other genes such as proteoglycan, collagen α_II and α_{II}I [2,39], EPC-1 [37,42] and cdc2 [43], it decreases during aging. For most of them the mechanisms of age dependent modulation are not clear. Age-dependent changes in DNA methylation patterns [44–46], chromatin structure [47,48], transcription factor activity [49], phosphorylation [50], and signal transduction [51,52] could explain some of the changes seen at the transcriptional level.

Our work describes that one key enzyme in signal transduction, PKC, shows an age dependent change both in activity and in protein expression. We find that the increase of total PKC activity and protein is mainly due to age specific changes of the PKC α−isoform, because expression of the other PKC isoforms present in fibroblasts remain unaltered during aging. Because it is known that PKC influences gene expression via the AP1 element, we analysed the expression of AP1 responsive genes that are known to alter during aging. Our data show that the expression of collagenase (MMP-1) increases in parallel with PKC α, both at the mRNA and protein level, strengthening the hypothesis that aging affects gene expression via PKC and AP1. It remains to be seen whether the link between increased PKC activity and collagenase expression is direct or indirect and whether other regulatory processes occur during fibroblasts aging. Similar to other groups, we find that the increase of collagenase (MMP-1) expression is not paralleled by a similar increase of its natural inhibitor TIMP-1, suggesting that it may lead to an age dependent collagen degradation [23]. It remains to be seen whether MMP-1 and TIMP-1 mRNA levels directly correlate with their activity. Since other inhibitors of collagenase (MMP-1) have been described, it is possible that the age dependent increase of collagenase expression is sufficiently counteracted [53] We furthermore describe that a natural inhibitor of PKC, α-tocopherol, reduces both PKC activity, and hence collagenase expression. In line with this, the PKC inhibitor H7 reduced collagenase expression in human skin fibroblasts [54] and, moreover, UV induced collagenase overexpression is inhibited by the tocopherol analogue trolox [55]. Whether α-tocopherol could also interfere with collagenase expression by acting as an antioxidant is currently under investigation. A scheme of the possible effects of α-tocopherol on skin aging are shown in Fig. 7.

Prolonged treatment of human diploid fibroblasts with α-tocopherol does not extend their life span in culture [56,57], but it prevents lipid peroxidation occurring during aging [58] and, as shown here, it modulates age dependent changes in gene expression. However, interference with aberrant PKC signal transduction by natural compounds such as α-tocopherol or other inhibitors could be a way to slow down processes occurring during skin aging. It could also interfere with other phenotypes such as skin ulcers in diabetic patients. Interestingly, the concentration of the physiological activator of PKC, diacylglycerol, is increased in diabetes, and a reduction of PKC activity in kidney mesangial cells in diabetic patients has been shown to occur after vitamin E treatment [59,60]. In these patients, an increase in PKC activity (due to increase diacylglycerol synthesis), followed by collagenase (MMP-1) expression and skin collagen degradation may play a concomitant role. In this case, skin treatment as well as systemic administration of

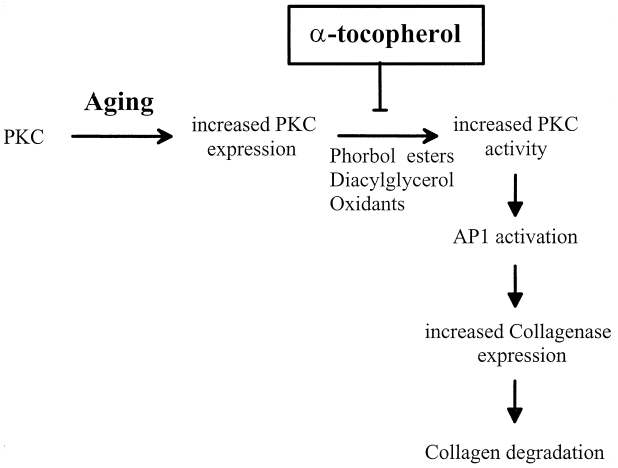

Fig. 7. Putative mechanism of α-tocopherol action in aging skin fibroblasts. Aging of fibroblasts leads to increased expression of protein kinase C (PKC) and to higher PKC activity, which is also increased by phorbol esters, diacylglycerol and environmental stress such as oxidants. The activation of downstream targets of PKC such as AP1 is facilitated, thus leading to higher expression of AP1-regulated genes such as collagenase (MMP-1) resulting in collagen degradation. α-Tocopherol possibly resets increased levels of PKC activity resulting from aging and environmental stresses, and thus may prevent collagenase overexpression and protect the skin from collagen degradation

α-tocopherol to diabetic patients may have beneficial results. Finally, oxidative stress caused by ultraviolet (UV) radiation, ozone, hydrogen peroxide and free radicals are known to increase PKC activity [61–63]. Skin damage by oxidants may lead to activation of PKC and AP1, thus increasing collagenase (MMP-1) expression and collagen degradation. Alpha-tocopherol protection of oxidant induced skin aging may be direct by eliminating radicals and oxidants, or indirect, by inhibiting PKC activity and AP1 activation. In line with this, it was recently suggested that retinoic acid may prevent photoaging by inhibition of c-jun protein induction thus reducing collagenase expression [64,65].

Finally, we can speculate whether an intrinsic cellular aging program exists that gives rise to events such as PKC α activation and overall collagenase overexpression, ultimately leading to collagen degradation. Superimposed upon this genetic program, environmental effects such as oxidants, UV radiation, increased blood glucose, toxic compounds and tumor promoters may create additional damage leading to PKC activation. α-Tocopherol inhibition of PKC may be a natural way to protect against skin aging and inflammatory conditions by resetting the level of expression of PKC. This protection may be part of the molecular basis for α-tocopherol action against age dependent skin damage, in addition to its well known antioxidative properties.

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