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β-Carotene interference with UVA-induced gene expression by multiple pathways*

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Abstract: UVA exposure causes skin photoaging by singlet oxygen ($^1O_2$)-mediated induction of matrix metalloproteases (MMPs). We assessed whether β-carotene, a carotenoid known as $^1O_2$ quencher and retinoic acid (RA) precursor, interferes with UVA-induced gene regulation and prevents UVA-induced gene regulation in HaCaT human keratinocytes. HaCaT cells accumulated β-carotene in a time- and dose-dependent manner. UVA irradiation massively reduced the cellular β-carotene contents. β-Carotene suppressed UVA induction of MMP-1, MMP-3, and MMP-10—three major MMPs involved in photoaging. HaCaT cells produced weak retinoid activity from β-carotene, as demonstrated by mild up-regulation of retinoid receptor RARβ and activation of an RARE-dependent reporter gene. Of the 568 UVA-regulated genes, β-carotene reduced the UVA effect for 143, enhanced it for 180, and did not interact with UVA for 245 genes. The pathways regulated β-carotene in interaction with UVA were characterized by genes involved in growth factor signaling, stress response, apoptosis, cell cycle, extracellular matrix (ECM) degradation, tanning, and inflammation.

In conclusion, β-carotene at physiological concentrations interacted with UVA effects by multiple mechanisms that included, but were not restricted to, $^1O_2$ quenching. With our results, we provide a mechanistic basis for the long-known and clinically established photoprotective effects of β-carotene in human skin.

Keywords: β-carotene; gene expression; UVA; photoaging; skin; singlet oxygen.

INTRODUCTION

More than 600 carotenoids are known in food, among those the major carotenoids that occur in human plasma are all-*E* β-carotene, α-carotene, β-cryptoxanthin, lycopene, and the xanthophylls lutein and zeaxanthin. There has been considerable interest in the carotenoids for many years owing to their radical scavenging and singlet oxygen quenching properties and thus their putative role in photochemistry, photobiology, and photomedicine [1]. There are several examples of how nature uses carotenoids for photoprotection: Photosynthetic bacteria, algae, and green plants utilize carotenoids as constituents of the photosynthesis reaction center for protection against photosensitization during light-harvesting processes. There is also a positive correlation between solar radiation exposure and the proportion of carotenoid pigmented bacteria in the outdoor atmospheric microbial populations, indicating that pigmentation is an efficient protection mechanism of microorganisms against solar light [2]. Carotenoid

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sequestration is also utilized in monarch butterflies containing high amounts of carotenoids in their caterpillars to protect them after consumption of phototoxic plants.

Solar light has been implicated in the photoaging process via UVA radiation (320–400 nm; UVA1 340–400 nm, UVA2 320–340 nm) [3]. UVA induces reactive oxygen species (ROS), including $^1O_2$, and UVA exposure is thought to cause skin aging mainly by $^1O_2$-dependent pathways. $^1O_2$ in turn can regulate the expression level of a variety of genes, including genes involved in photoaging. $^1O_2$-mediated gene induction has been shown for matrix metalloprotease-1 (MMP-1) [4], heme oxygenase-1 [5], interleukin-1 (IL-1) and 6 (IL-6), as well as for ICAM-1 (Intercellular Adhesion Molecule 1) [6]. $^1O_2$ mediates gene regulation via the transcription factor AP-2 [7]. Furthermore, like UVB/UVA2, UVA1 activates stress-activated protein kinases. Inhibition or moderation of these molecular events could confer photoprotection on target cells. An overview on the effects of UV light is shown in Fig. 1.

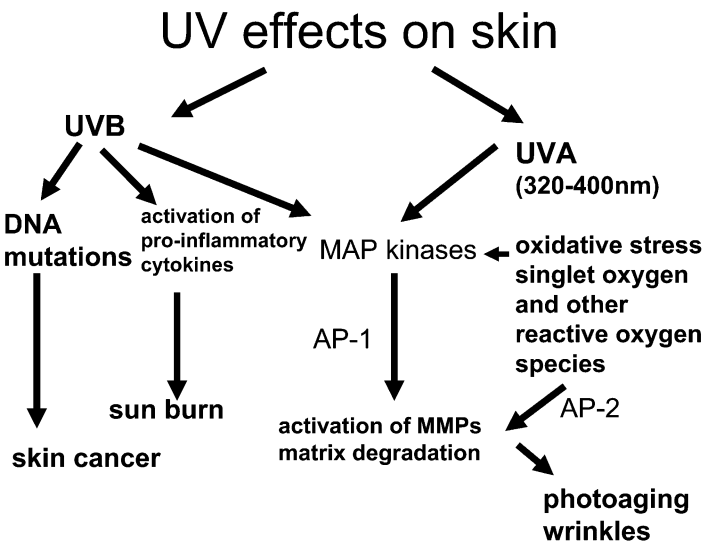

Fig. 1 Overview of major UV effects in skin.

β-Carotene and other carotenoids are transported to the skin and accumulate mainly in the epidermal layers [8]. β-Carotene has the potential to protect skin firstly, because it is an excellent $^1O_2$ quencher [9]. During UVA exposure, skin is regularly exposed to $^1O_2$, and is thus a most relevant tissue to test $^1O_2$ quenching by β-carotene in living cells. Secondly, β-carotene scavenges ROS other than $^1O_2$ [10]. Through $^1O_2$-quenching, β-carotene reduces UVA-induced heme oxygenase 1 [11] and suppresses the formation of UVA-induced photoaging-associated mitochondrial DNA deletions [12] in human skin fibroblasts. Thirdly, β-carotene was established as a therapeutic of erythropoetic hematoporphyria, a disease in which the pathogenesis involves $^1O_2$ generation [13–15]. Finally, β-carotene has been shown to mildly reduce sun burn. β-Carotene can be metabolized to retinoic acid (RA), a signaling molecule involved in skin maintenance. Indeed, β-carotene metabolism to retinol has been shown in cultures of human skin fibroblasts, melanocytes, and keratinocytes, which take up β-carotene and increase their intracellular retinol concomitantly. The efficacy of topical tretinoin (all-*trans*-RA) in treating photoaging is well established. RA acts by stimulating the proliferation of keratinocytes, while inhibiting terminal keratinocyte differentiation. As a result, the thickness of the transit-amplifying (TA) keratinocyte layer in the epidermis is increased, leading to a smoother appearance of the skin. Moreover, RA can prevent UV induction of MMP-1, and UV repression of dermal collagen expression. (Collagenases, MMPs) play a major role in photoaging by degrading collagen and elastin in the dermis.

This leads to accumulation of fragmented and immature collagen fibers, loss of elasticity, and elastosis (see refs. [16,17]).

The goal of our studies was to elucidate the molecular pathways that are involved in the efficacy of β-carotene to protect skin from UVA-induced photoaging.

METHODS

All methods are described in detail in [16,17]. For all experiments, we used the well-characterized HaCaT human keratinocytes cell system. We chose keratinocytes as a model to analyze the β-carotene effect on UVA-induced gene regulation, since β-carotene is accumulated to a higher concentration in the epidermis, as compared to the dermis. In addition, keratinocytes in the epidermis are exposed to oxidizing UVA to a significant extent over their life span. Thus, we consider HaCaT keratinocytes a meaningful model to analyze how β-carotene regulates UVA-induced gene expression. HaCaT keratinocytes were pretreated for 2 d with β-carotene at 0.5, 1.5, and 3.0 μM, typical concentrations in human plasma after low-to-moderate dietary supplementation [18]. Subsequently, the cells were irradiated with solar simulated light (SSR) consisting of UVA1/2 as major light spectrum—further referred to as UVA. The dose of UVA radiation applied is equivalent to that experienced on a sunny day at 35 N in 1–2 h. We investigated whether β-carotene is able to alleviate $^1O_2$-mediated MMP induction by UVA in a physiological dose range. mRNA expression of MMPs was analyzed by TaqMan® real-time polymerase chain reaction (RT-PCR). Moreover, we attempted to differentiate the $^1O_2$-mediated effects in interaction with UVA from those associated with its provitamin A function. Therefore, we analyzed the cells by high-pressure liquid chromatography (HPLC) for the formation of metabolic or degradation products with retinoid activity, and correlated the presence of such metabolites with RA-dependent gene regulation. The identification of the major β-carotene metabolites formed in cells was based on expected elution order as well as on absorption spectra obtained by photodiode array detection. To confirm these results, some cell extracts were analyzed by APCI+ tandem mass spectrometry. The main metabolites formed were identified as (13*Z*)-β-carotene, 4'-β-apocarotenal, 8'-β-apocarotenal, and monoepoxy-β-carotene. Since the expected amount of RA was below the limit of detection, we used an RARE-driven reporter construct to indirectly measure retinoid activity.

Using transcriptomics (Affymetrix GeneChip® analysis), we analyzed how β-carotene influences the response of HaCaT cells to UVA exposure. In addition, we analyzed the effect of β-carotene in unirradiated cells. Five independent, factorially designed cell irradiation experiments were analyzed by microarray hybridization. For each experiment, one chip was hybridized per treatment condition. GeneChip® analysis was done as described [19]. Gene regulations by β-carotene and/or UVA were calculated relative to placebo. Changes in gene expression were only included in further analysis, if the change factor was ≥0.5 or ≤–0.5, and if unpaired t-tests yielded p values ≤0.05. To identify pathways affected by the treatments, functional information on the genes was retrieved from public literature databases.

SUMMARY OF RESULTS

β-Carotene inhibits expression of photoaging-associated MMPs

Since $^1O_2$-dependent induction of MMPs upon UVA exposure is thought to be a major mechanism of photoaging, we analyzed whether β-carotene would inhibit MMP induction upon UVA exposure. Among MMPs, MMP-1 is best characterized in terms of induction by UV light, and is the most accepted marker for photoaging. The degree of UVA inducibility of MMP-1 expression varied between experiments. In any case, β-carotene at a concentration of 1.5 μM significantly reduced UVA-induced MMP-1 induction from 1.3- to 0.9-fold on average (Fig. 2a). Down-regulation of UVA-induced MMP-1 production by β-carotene was also confirmed on the protein level (data not shown). UVA also induced

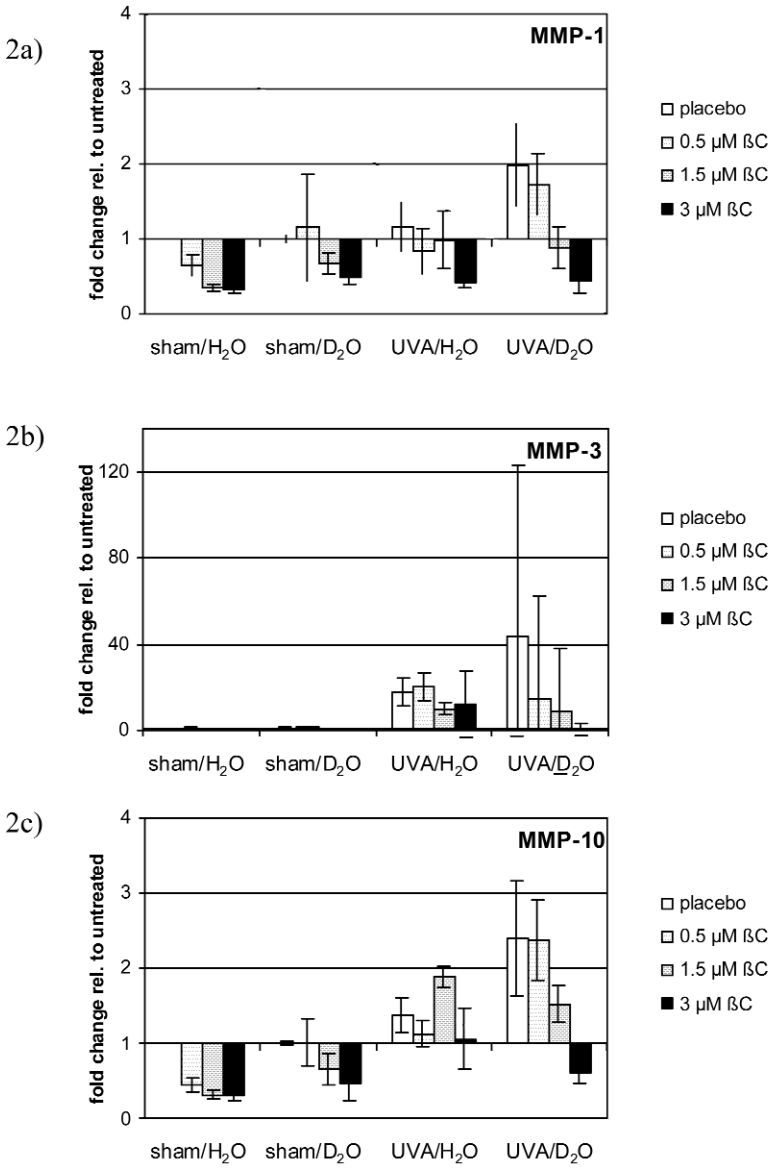

Fig. 2 D_2O treatment enhanced UVA induction of (a) MMP-1, and (c) MMP-10, indicative of a $^1O_2$-dependent mechanism. β-Carotene quenched UVA/D_2O-induced expression of MMP-1, (b) MMP-3, and MMP-10: HaCaT cells were pretreated for 2 d with 0.5, 1.5, or 3 µM β-carotene. The cells were irradiated with UVA (270 kJ/m^2) either in D_2O-containing PBS or in H_2O-containing PBS, to analyze $^1O_2$ inducibility of genes. Gene expression 5 h after UVA irradiation was analyzed by quantitative real-time PCR (QRT-PCR). The graphs present data from three independent experiments. Error bars indicate standard error. D_2O enhanced MMP1 induction by UVA from 1.2- to 1.9-fold, MMP-3 induction from 18- to 43-fold (not significant), and MMP-10 induction from 1.4- to 2.4-fold. β-Carotene dose-dependently suppressed UVA/D_2O induction of MMP-1, MMP-3, and MMP-10 to below expression levels in unirradiated controls at the highest concentration (3 µM).

MMP-10 expression to ca. 3-fold relative to the expression in unirradiated cells (Fig. 2c). 1.5 µM β-carotene reduced UVA induction of MMP-10 to approximately 2.5-fold. MMP-3 (stromelysin-1) was analyzed as a close relative to MMP-10. MMP-3 is also known to be induced by UVA1 light

(340–450 nm). Accordingly, MMP-3 was induced approximately 49-fold by UVA exposure, and 1.5 μM β-carotene reduced UVA induction of MMP-3 to 27-fold relative to unirradiated controls (Fig. 2b).

Furthermore, we analyzed the expression profiles of the two gelatinases MMP-2 and MMP-9, the latter of which is induced by UV irradiation in skin. Unexpectedly, neither of the gelatinases was induced by our irradiation regimen, and β-carotene did not influence their expression significantly (data not shown). TIMP-1, an endogenous MMP inhibitor, was strongly expressed but not significantly influenced by the treatments.

We also investigated whether the mechanism, by which β-carotene interferes with UVA induction of MMPs, involves $^1O_2$ quenching. To test this, we performed experiments, in which cells were irradiated either in D_2O-containing buffer or in H_2O-containing buffer. D_2O is able to prolong the lifetime of $^1O_2$ [18]. Thus, the probability of $^1O_2$ to react with a relevant target is increased. Accordingly, $^1O_2$-dependent MMP induction upon UVA exposure should be more pronounced after irradiation in the presence of D_2O. β-Carotene should then be able to reduce MMP induction by UVA/D_2O treatment.

In line with this hypothesis, β-carotene significantly and dose-dependently reduced UVA/D_2O-induced MMP-1 induction at 1.5 and 3 μM. Moreover, β-carotene treatment also tended to reduce basal MMP-1 RNA in unirradiated cells (Fig. 2a). Although MMP-10 is known to be induced by UV light, it had not been demonstrated whether its regulation also involves $^1O_2$-dependent pathways. D_2O significantly enhanced UVA induction of MMP-10 from 1.4- to 2.4-fold relative to unirradiated controls (Fig. 2c). This shows that MMP-10 induction by UVA irradiation involves $^1O_2$-dependent mechanisms. Pretreatment of cells with different doses of β-carotene opposed MMP-10 induction by UVA and D_2O in a dose-dependent fashion. As for MMP-1, β-carotene also tended to reduce the basal MMP-10 expression in unirradiated cells.

MMP-3 induction by UVA was enhanced by irradiation in D_2O-containing buffer from 18- to 43-fold (Fig. 2b). β-Carotene prevented MMP-3 induction by UVA irradiation in the presence or absence of D_2O.

β-Carotene metabolism and retinoid activity in HaCaT skin keratinocytes and effects of UVA

β-Carotene was time-dependently accumulated in HaCaT cells. The β-carotene concentration in cells was dose-dependent, and increased from 63 pmol/million cells at 0.5 μM to 406 pmol/million cells at 3.0 μM within a culture period of 72 h. UVA irradiation diminished the β-carotene stores to ca. 13 % in cells incubated in 1.5 or 3 μM β-carotene (Fig. 3).

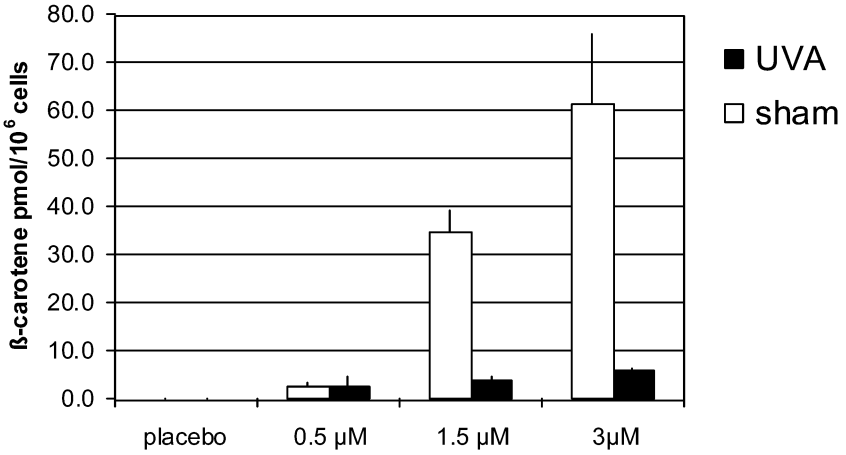

Fig. 3 UVA irradiation depleted cellular ß-carotene stores: HaCaT cells were supplemented with 0.5, 1.5, or 3 µM β-carotene for 2 d prior to UVA (270 kJ/m^2) irradiation. Cellular β-carotene contents were analyzed by HPLC. Error bars indicate standard deviation. The graph shows data from an experiment with three replicates. UVA irradiation diminished the β-carotene stores to ca. 13 % in cells incubated in 1.5 or 3 µM β-carotene. UVA did not reduce the cellular β-carotene content after incubation with 0.5 µM β-carotene.

By HPLC analysis, we found that HaCaT keratinocytes do not produce detectable amounts of retinol or retinyl esters from β-carotene (Table 1). In contrast, apocarotenals were detected. The cellular apocarotenal contents increased dose dependently, and amounted to maximum 5 pmol/million cells treated with 3 µM β-carotene. Moreover, a fraction of the supplemented all-*E* β-carotene was isomerized to (*Z*)-isomers. The amount of (*Z*)-isomers also increased dose-dependently, and was maximum 0.8 pmol/million cells after supplementation with 3 µM β-carotene.

Table 1 β-Carotene uptake and metabolism in HaCaT cells: HaCaT cells were treated with 0.5, 1.5, or 3 µM β-carotene for 2 d. Cellular contents of β-carotene and β-carotene metabolites was quantified by HPLC. <LOD: below limit of detection.

β-Carotene supplementation (µM)	all-*E*-β-Carotene (pmol/10e6 cells)	(*Z*)-β-Carotene (pmol/10e6 cells)	Apocarotenals (pmol/10e6 cells)	Retinol (pmol/10e6 cells)	Retinyl palmitate (pmol/10e6 cells)
Placebo	<LOD	<LOD	<LOD	<LOD	<LOD
0.5	9.7 ± 0.09	0.2 ± 0.07	1.18 ± 0.04	<LOD	<LOD
1.5	34.3 ± 0.05	0.41 ± 0.02	3.21 ± 0.19	<LOD	<LOD
3.0	63.90 ± 0.22	0.82 ± 0.16	5.04 ± 0.11	<LOD	<LOD

Despite undetectable retinol formation from β-carotene, RA was formed after β-carotene treatment, as shown by transactivation of an RA-dependent reporter gene (Fig. 4). Treatment of HaCaT cells with 1 or 3 µM β-carotene caused activation of the luciferase reporter to a degree comparable to what was achieved after treating the cells with a combination of all-*trans*-RA and 9-*cis*-RA at 10 nM each. RARE-dependent gene activation by β-carotene was reduced to ca. 70 %, if the cells were irradiated with UVA prior to the activation measurement.

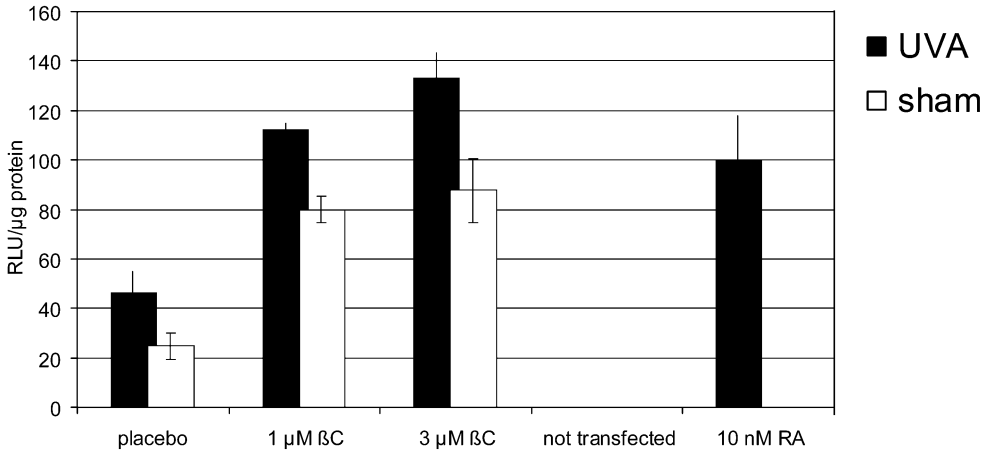

Fig. 4 β-Carotene at 1 or 3 µM transactivates an RA-dependent reporter construct to a degree comparable to 10 nM all-*trans*-RA: HaCaT cells were transiently transfected with the luciferase reporter construct. Luciferase activity was determined after 40 h treatment with β-carotene. The graph represents two experiments with four replicates each. Error bars indicate standard error. Relative to placebo, the RARE-driven reporter gene was induced 2.4- and 2.9-fold by 1 and 3 µM β-carotene, respectively. In comparison, 10 nM RA induced the reporter construct 2.2-fold. Consistent with the finding that UVA irradiation depletes cellular β-carotene contents, transactivation of the reporter gene was decreased to 53–71 % in irradiated cells.

We correlated our results on β-carotene metabolism and RA-dependent gene activation with the expression profiles of the two β-carotene cleavage enzymes and the nuclear receptors responsible for transducing the RA effect on gene expression. β-Carotene-15,15'-oxygenase cleaves β-carotene centrally to yield retinal. β-Carotene-15,15'-oxygenase was expressed at a relatively low level in controls. Transcripts for β-carotene-9',10'-oxygenase, which produces 10'-apocarotenal and β-ionone from β-carotene, were present at a ca. 23-fold higher abundance. The RNA levels of both enzymes were not significantly influenced by treatments.

Expression of all six retinoid receptor (RAR) genes was detected in HaCaT cells. UVA down-regulated all retinoid receptors approximately 2-fold, except for RARα, which was not influenced by UVA. Apparently, regulation of RARα and γ expression, as well as regulation of RXRα and γ has a $^1O_2$-dependent component, as D_2O treatment had a significant effect on these transcripts. β-Carotene had no significant effect on the basal or UVA-regulated expression levels of RARs and RXRs. Of note, β-carotene nonsignificantly induced RARβ in a dose-dependent manner, an effect observed predominantly in unirradiated cells (Fig. 5). It shows that weak retinoid activity is formed from β-carotene in HaCat cells, which may be explained by the eccentric cleavage products of β-carotene, apocarotenals, present at detectable concentrations in HaCaT cells. Although apocarotenals can also be formed by oxidative breakdown, their prevalence is in accordance with the higher expression of the eccentric cleavage enzyme β-carotene-9',10'-oxygenase. Apocarotenals can be metabolized to RA via β-oxidation, and may well serve as the precursors of the RA that was indirectly detected by monitoring gene regulation.

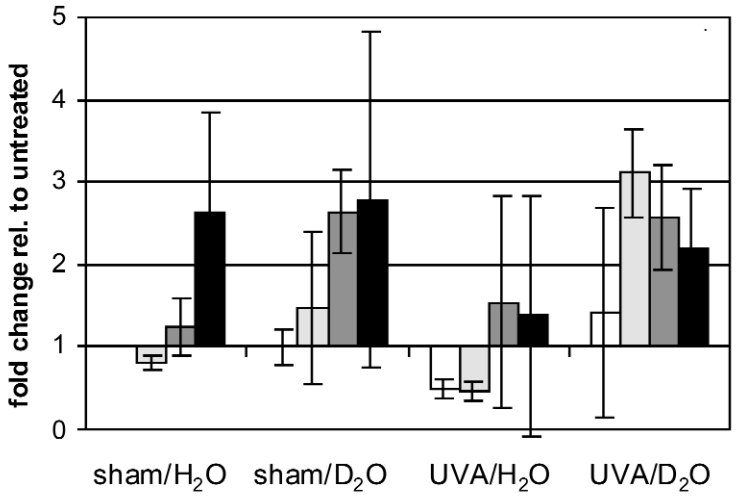

Fig. 5 Effect of β-carotene on expression of retinoid receptor (RAR) β2 after UVA or D_2O-enhanced UVA irradiation: HaCaT cells were pretreated for 2 d with 0.5, 1.5, or 3 μM β-carotene. The cells were irradiated with UVA (270 kJ/m^2) either in D_2O-containing PBS or in H_2O-containing PBS, to analyze $^1O_2$ inducibility of the gene. Gene expression 5 h after UVA irradiation was analyzed by QRT-PCR. β-Carotene mildly up-regulated RAR β2. RARα and γ, and RXR α, β, γ expression was also decreased by UVA, but none of them influenced by β-carotene (data not shown).

Effects of β-carotene on UVA-induced gene expression

In unirradiated keratinocytes, β-carotene reduced expression of immediate early genes (e.g., GEM, KRS-2, JUN-B, FRA-2, EGFRα), indicating reduced stress signals. Moreover, gene regulations by β-carotene suggest decreased extracellular matrix (ECM) degradation, e.g., inhibited MMP-10 expression, and increased keratinocyte differentiation. This effect on differentiation was unrelated to UVA exposure, but synergized with UVA effects.

Down-regulation of growth factor signaling, moderate induction of proinflammatory genes, up-regulation of immediate early genes, including apoptotic regulators and suppression of cell cycle genes, were hallmarks of the UVA effect. Of the 568 UVA-regulated genes, β-carotene reduced the UVA effect for 143, enhanced it for 180, and did not interact with UVA for 245 genes. The different interaction modes imply that β-carotene/UVA interaction involved multiple mechanisms.

In UVA-irradiated cells, β-carotene inhibited gene regulations by UVA, which promote ECM degradation, arguing for a photoprotective effect of β-carotene. β-Carotene-enhanced UVA-induced PAR-2 expression, suggesting that β-carotene enhances tanning after UVA exposure. The combination of β-carotene-induced differentiation with the cellular "UV response" led to a synergistic induction of cell cycle arrest and apoptosis by UVA and β-carotene. A summary of the most important pathways and genes regulated is given in Table 2.

Tables 2a and b Overview on major pathways regulated and transcriptional response to β-carotene (βC) and/or UVA treatment. HaCaT human keratinocytes were pretreated for 2 d with βC at 1.5 μM/L. Subsequently, the cells were irradiated with SSR consisting mainly of UVA. Oligonucleotide array hybridization was performed for five independent experiments. For every gene represented on the chip, the average change induced by the treatments was calculated in relation to placebo-treated controls. All genes listed were significantly ($P < 0.05$) regulated in at least one treatment by a change factor greater than 0.5 or less than –0.5. The change factor given corresponds to the arithmetic mean of 5 replicates per condition. Up-regulations by a change factor of greater than 0.5, and down-regulations by a change factor of less than –0.5 are boldfaced.

2a

Accession number	Pathway and related genes	Fold changes in gene mRNA expression		
	Immediate early genes	βC	UVA	UVA & βC
AL022312	ATF4; activating transcription factor 4	0.11	**0.76**	**0.96**
V01512	C-FOS	–0.14	**0.81**	**2.49**
X16707	FRA-1	0.14	**0.61**	**0.71**
X51345	JUNB	**–0.5**	–0.28	**–0.7**
X56681	JUND	0	**1.17**	**1.66**
U13045	NRF2, subunit beta 1	**–1.54**	–0.42	–0.11
S62138	GADD153	–0.45	**4.11**	**7.64**
Z50194	TDAG51; PQ-rich protein; PHLDA1	0.27	**2.3**	**6.19**
U83981	GADD34	–0.11	**1.16**	**2.62**
AF050110	TIEG, EGRα	**–0.81**	0.3	–0.11
S81914	IEX-1	–0.08	**1.65**	**0.91**
	Extracellular matrix			
X07820	MMP10	**–1.26**	**3.59**	**2.2**
	Inflammation			
	VEGF-related ligands and receptors			
AF022375	VEGF	**–0.51**	**1.1**	**0.78**
	IFNα/β			
M14660	IFIT2; ISG-54K; (interferon stimulated gene)	0.18	**2.09**	**1.17**
L05072	IRF-1; interferon regulatory factor 1	0.16	**0.77**	0.29
	Interleukins			
X04430	IL6; Interleukin 6; IFN-beta 2a	**0.63**	**0.65**	**2.29**
	EGF-related ligands and receptors			
M60278	HB-EGF; heparin-binding egf-like growth factor	0.33	**1.53**	**3.32**
X00588	EGFR; precursor of epidermal growth factor receptor	**–0.56**	–0.34	**–0.69**
	Wnt signalling			
l20861	WNT5A	–0.47	**–0.68**	**–2.22**
l20861	WNT5A	**–1.7**	**–1.53**	**–3.34**
l37882	frizzled-2	–0.03	–0.27	**–1.38**
AB012911	frizzled-6	**–0.52**	**–0.73**	**–1.16**

2b

Accession number	Pathway and related genes	Fold changes in gene mRNA expression		
	Differentiation markers	βC	UVA	UVA & βC
M21389	Keratin 5	**0.86**	0.11	**0.81**
J00124	Keratin 15	**1.02**	–0.11	**0.96**
M28439	Keratin 16	**–1.97**	0.08	–0.38
M91669	Bullous pemphigoid autoantigen bp180	–0.45	0.07	**–0.57**
X53586	Integrin α6	**–1.06**	0	**–0.76**
U40282	ILK; integrin-linked kinase	–0.23	–0.13	**–0.51**
M58526	Collagen type IV, α-5 (COL4A5)	**–0.83**	**–1.11**	**–1.16**
L02870	Collagen type VII, α-1 (COL7A1)	–0.21	–0.15	**–0.83**
U70663	KLF4; EZF (epithelial Zn finger)	–0.17	**1.99**	**3.46**
	Cell cycle			
	G1 phase			
M73812	Cyclin E	–0.23	**1.6**	**1.75**
X16277	Ornithine decarboxylase	–0.32	**0.73**	0.37
L49229	RB1	**–0.77**	**-0.8**	**–1.13**
U49844	ATR	–0.41	–0.21	**–0.53**
L78833	BRCA1	–0.13	–0.46	**–0.89**
X65550	mKI67a mrna (long type) for antigen of monoclonal antibody KI-67	–0.17	**–0.95**	**–1.59**
Z15005	CENP-E	–0.37	**–1.36**	**–1.44**
	Apoptosis			
U19599	BAXd	**0.55**	–0.23	**0.67**
AB020735	ENDOGL-2	**0.83**	0.35	0.41
U83857	AAC11	**–0.57**	–0.19	–0.05
	Retinoic acid target genes			
AF061741	RETSDR1; retinal short-chain dehydrogenase/reductase	**1.07**	**–0.93**	–0.06
X59373	HOXD4	**–0.52**	**–0.79**	**–0.76**
AF017418	MEIS2	0.26	**–1.39**	**–1.3**
U37146	SMRT	–0.33	**–0.52**	**–1.22**

The retinoid effect of β-carotene, as indicated by the expression profiles of RA target genes, was minor, indicating that the β-carotene effects reported here were predominantly mediated through vitamin A-independent pathways.

A model of the interactions of β-carotene and UVA is shown in Fig. 6. We propose that β-carotene reduced UVA induction of genes involved in ECM degradation and inflammation as a $^1O_2$ quencher. We suggest that the mild photoprotective effect of β-carotene is based on inhibition of these $^1O_2$-induced gene regulations, rather than on a physical filter effect, since its absorption maximum lies outside the UVB/UVA range at around 460 nm. β-Carotene, if scavenging ROS other than $^1O_2$, is irreversibly damaged and converted into radicals, if not rescued by other antioxidants. Thus, β-carotene did not inhibit UVA-induced stress signals, and enhanced some. UVA exposure suppressed several RA target genes. Since HaCaT cells produce marginal amounts of retinoid activity from β-carotene, the provitamin A activity of β-carotene did not translate into restored expression of RA target genes in this system.

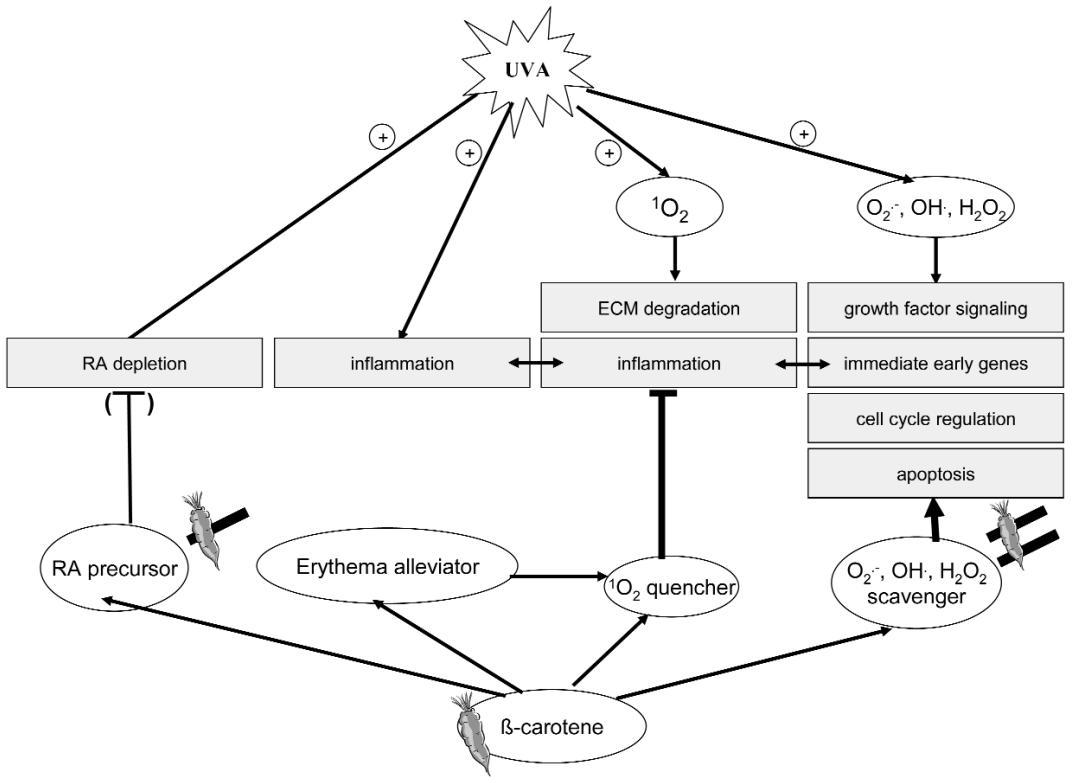

Fig. 6 Proposed relationship of the modes of action of β-carotene to its influence on UVA-induced biological processes.

SUMMARY AND CONCLUSION

HaCaT cells accumulated β-carotene in a time- and dose-dependent manner. UVA irradiation massively reduced the cellular β-carotene contents. In view of the role of vitamin A in maintaining skin integrity, depletion of vitamin A and provitamin A stores by UV light calls for an awareness of an increased vitamin A requirement in situations of extensive sun exposure. β-Carotene suppressed UVA induction of MMP-1, MMP-3, and MMP-10, three major MMPs involved in photoaging. We show that not only MMP-1, but also MMP-10 regulation involves $^1O_2$-dependent mechanisms. β-Carotene dose-dependently quenched $^1O_2$-mediated induction of MMP-1 and MMP-10. Thus, like in chemical solvent systems, β-carotene quenches $^1O_2$ also in living cells.

HaCaT cells produced weak retinoid activity from β-carotene, as demonstrated by mild up-regulation of RARβ and activation of an RARE-dependent reporter gene. UVA down-regulated all retinoid receptors, except RARα. β-Carotene did not regulate the genes encoding other RARs, RXRs, or the two β-carotene cleavage enzymes.

Transcriptomics analysis showed that β-carotene at physiological concentrations interacted with UVA effects in keratinocytes by multiple mechanisms that included, but were not restricted to, $^1O_2$ quenching.

Our results explain and integrate many conflicting reports on the efficacy of β-carotene as a $^1O_2$ quencher and as a general antioxidant in living cells. The identified mechanisms, by which β-carotene acts on the skin have implications on skin photoaging, as well as on relevant skin diseases, such as skin cancer and psoriasis. With these results, we also provide a mechanistic basis for the long-known and clinically established photoprotective effects of β-carotene in human skin.

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