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ORIGINAL RESEARCH

Chum salmon egg extracts induce upregulation of collagen type I and exert antioxidative effects on human dermal fibroblast cultures

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Abstract: Components of fish roe possess antioxidant and antiaging activities, making them potentially very beneficial natural resources. Here, we investigated chum salmon eggs (CSEs) as a source of active ingredients, including vitamins, unsaturated fatty acids, and proteins. We incubated human dermal fibroblast cultures for 48 hours with high and low concentrations of CSE extracts and analyzed changes in gene expression. Cells treated with CSE extract showed concentration-dependent upregulation of collagen type I genes and of multiple antioxidative genes, including OXR1, TXNRD1, and PRDX family genes. We further conducted in silico phylogenetic footprinting analysis of promoter regions. These results suggested that transcription factors such as acute myeloid leukemia-1a and cyclic adenosine monophosphate response element-binding protein may be involved in the observed upregulation of antioxidative genes. Our results support the idea that CSEs are strong candidate sources of antioxidant materials and cosmeceutically effective ingredients.

Keywords: fish egg, antiaging, gene expression analysis, antioxidative gene, phylogenetic footprinting analysis

Introduction

Skin is the body's largest organ, representing the interface between self and nonself. Skin aging is most prominently caused by extrinsic factors, mainly the permanent exposure of this organ to oxidative environmental stimuli, such as solar radiation, cigarette smoke, and other pollutants.[1] The second most important contributor to skin aging is an intrinsic factor: the age-related mitochondrial enzyme dysfunction that inhibits epidermal regeneration.[2]

In the field of cosmetic science, skin aging is clinically characterized by water loss, reduced skin thickness, sagging, and wrinkle formation.[3] At the molecular level, skin aging is characterized by reduced procollagen synthesis[4–6] and degradation of the extracellular matrix,[7] which mainly comprises collagen, glycosaminoglycan, and elastin. Aged skin fibroblasts become detached from the destabilized extracellular matrix, leading to a rounded and collapsed appearance. This in turn upregulates matrix metalloproteinase expression, activating a positive-feedback loop that further accelerates collagen matrix degradation.[8] Based on evidence that oxidative stress plays pivotal roles in both intrinsic and extrinsic aging, it has been suggested that antioxidants may be an efficient means of defense against aging processes.[9–11]

Numerous studies have examined compounds derived from marine and botanical organisms that show cosmetically useful antioxidant and antiaging activities.[12,13]

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Fish roe is a potential source of antiaging material, as it contains vitamins, proteins, unsaturated fatty acids, etc.[14] In a previous study, Marotta et al[15] demonstrated that nutraceuticals derived from beluga sturgeon's caviar (containing DNA, collagen elastin, and protein) robustly promoted the expression of collagen type I following in vitro supplementation.

In this study, we investigated the cosmetic use of chum salmon eggs (CSEs), called "Ikura" in Japanese. Differentiating them from other fish eggs, CSEs exhibit high concentrations of proteins (27%–35% of total weight) and crude lipids (12%–20%), with 30% of the total lipids comprising phospholipids and 63% triglycerides. CSEs also show high content of vitamin A (50–3,000 IU/g),[14] which is one of the most popular skin aging treatments.[16] The xanthophyll carotenoid astaxanthin is the major contributor to the deep yellow-orange color of the muscle of salmon and is also found in CSEs. Astaxanthin is the precursor of vitamin A[17] and per se quenches free radicals without being destroyed or becoming a pro-oxidant in the process.[18] Using a repeated insult patch test, randomized clinical trials have shown improvements in several important parameters for perception of skin facial health and aging, following treatment with extract from eggs of Atlantic salmon[19] (belongs to the same Salmonidae family as chum salmon). However, the effectiveness of CSEs in vitro has not yet been ascertained.

Here, we tested the efficacy of CSE extract using collagen synthesis ability as a measure of skin rejuvenation capacity. We also examined the alterations of gene expression related to oxidative stress response and reactive oxygen species (ROS) metabolism.

Materials and methods

Salmon egg extract preparation

The ethics committee of Yokohama City University does not require approval to be sought for experiments with commercial cells. Raw CSEs (purchased online) were homogenized using the TissueRuptor rotor-stator device (Qiagen NV, Venlo, the Netherlands) and were diluted with phosphate-buffered saline to a concentration of 10^5 mg/L. This solution was centrifuged at 1,200 rpm for 5 minutes, and the supernatant was passed through a 0.22 μm filter. CSE solutions were stored at −80°C and, once thawed, were either used up or discarded.

Cell culture

Normal human neonatal skin fibroblasts (NB1RGB cells) were purchased from the RIKEN Cell Bank (Ibaraki, Japan). These cells were cultured in α-MEM medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), penicillin 100 U/mL, and streptomycin 2.5 μL/mL. Cells were plated at a concentration of 1×10^5 cells/dish (100 mm φ), and the plates were supplemented with CSE solution at 80 μg/mL or 800 μg/mL or with phosphate-buffered saline as a negative control. Each experimental condition was replicated two times.

After incubation for 48 hours at 37°C in humidified air with 5% CO_2, total RNA was isolated from fibroblasts using TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. Extracted RNA was stored at −80°C.

Gene expression assay

Reverse transcription of total RNA was conducted using SuperScript II Reverse Transcriptase (Thermo Fisher Scientific), and the product was stored at 4°C. Quantitative real time-polymerase chain reaction (RT-PCR) of the collagen 1A1 and 1A2 genes was performed using the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific) with TaqMan gene expression assays. Relative quantification of gene expression was accomplished using the $2^{-\Delta\Delta Ct}$ method, with glyceraldehyde-3-phosphate dehydrogenase gene as an endogenous control.

Oxidative stress analysis PCR array

After confirming the fold changes of collagen genes, we assessed the total antioxidant profile of the cells using Human Oxidative Stress RT^2 Profiler PCR Array (Qiagen NV), which contains 84 key genes related to the oxidative stress response. All reactions were carried out using the StepOnePlus Real-Time PCR System according to the manufacturer's protocol. Relative expression values were calculated using the $2^{-\Delta\Delta Ct}$ method. Genes with low absolute expression levels were excluded from further analyses, and the cut-off was set at 35 Ct.

All values from both the TaqMan assay and PCR array experiments are presented as the arithmetic means of two biological replicates.

Promoter analysis in silico

We performed transcription regulation analysis of 33 genes related to the oxidative stress response with fold changes over the defined threshold (induced) and of 31 genes with expression changes below the threshold (uninduced) in the 800 μg/mL CSE condition. The Sequence Homology in Higher Eukaryotes (in preparation) tool was used to perform the comparative genome promoter analysis in the human, mouse, and rat genome.[20] We used sequences to 5,000 nucleotides upstream and 200 nucleotides downstream from the transcriptional start sites referenced in the DBTSS database.[21] Analysis included the following

steps:[20] 1) pairwise (human–mouse and human–rat) alignment (≥50% similarity); 2) multiple alignment of human, mouse, and rat promoters; 3) computing the multiple alignment score[22] for alignments conserved among three species in order to assign the strength of the identified orthologous alignment compared to a nonorthologous alignment; 4) scoring the human sequence with publicly available Transfac[23] matrices; and 5) selecting the best scoring motifs using the Pareto front method.[24] Additionally, we compared the transcription factor frequencies using the motif conservation score[25] for the induced gene set and for the uninduced gene set. Finally, transcription factors with motif conservation score ≥10 were chosen as candidates for the transcription regulation of CSE-induced oxidative stress genes.

Composition analysis of CSEs

In order to presume what triggered observed changes involving multiple genes and transcription factors, we conducted a content analysis of CSEs, which was outsourced to a specialized institution, Japan Food Research Laboratories (Tokyo, Japan). The content of protein was quantified by the Kjeldahl method, and the total lipid content was measured by the acid hydrolysis method. The quantities of vitamin A, vitamin E, and astaxanthin were determined by high performance liquid chromatography (HPLC) method.

Results

TaqMan assay revealed that supplementation with CSE extracts led to concentration-dependent upregulation of collagen type I mRNA expression (Figure 1). COL1A1 expression was 125% of the control expression level with CSEs of 80 µg/mL and 211% with CSEs of 800 µg/mL. Similarly, COL1A2 expression was 136% of the control expression level with CSEs of 80 µg/mL and 262% with CSEs of 800 µg/mL.

Oxidative stress PCR array analysis

Analysis of the oxidative stress pathway with the RT^2 Profiler PCR Array revealed that 64 of the 84 genes were expressed in all samples, and we were able to obtain their meaningful relative expression values. To define a "prominent" increase or decrease in gene expression, we set the fold-change threshold to ±2. Table 1 lists the genes showing prominent up- or down-regulation, excluding those with low absolute expression levels, ie, for which the average threshold cycle is relatively high (>30) in the control, 80 µg/mL, or 800 µg/mL condition. We found upregulation of many genes related to the oxidative stress response, with the greatest fold change observed

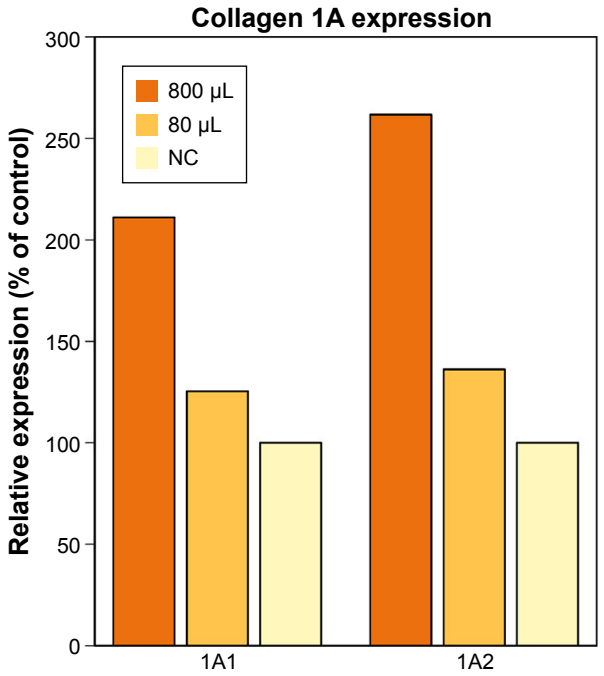

Figure 1 Expression of collagen type I gene with CSE extract supplementation relative to negative control.
Abbreviations: CSEs, chum salmon eggs; NC, negative control.

for the peroxiredoxin family genes (PRDX3, PRDX4, and PRDX5), thioredoxin (TXN) reductase 1 (TXNRD1), and oxidation resistance 1 (OXR1). We also observed notably high fold changes of PRDX1 (24.88 with 80 µg/mL and 3.01 with 800 µg/mL); however, it was omitted from Table 1 due to its relatively low absolute expression levels.

The peroxiredoxin system and the glutathione peroxidase (GPX) system are the two main sets of oxidative stress response genes. Compared to the peroxiredoxin system genes, GPX system genes showed less prominent relative expressions, with most genes showing a change of only −1.5- to 1.5-fold. No GPXs (GPX1–GPX7) were highly upregulated, but several glutathione-related genes were prominently upregulated with 800 µg/mL CSEs, including MGST3 (2.16-fold), GCLM (2.14-fold), and GSTP1 (2.12-fold).

Other major ROS metabolism genes, including catalase and superoxide dismutases (SOD1–SOD3), also did not exhibit prominent upregulation. SOD3 showed upregulated expression (2.15 in 80 µg/mL and 1.52 in 800 µg/mL), but its absolute expression was below the criteria for inclusion in Table 1.

The other prominently increased genes showed diverse primary roles, including peroxidase activity (PXDN), stabilizing proteins against aggregation (HSPA1A), iron storage (FTH), reduction of antioxidant molecules (NQO1), and antioxidant molecule preparation (HMOX1). However,

Table 1 Differentially regulated genes involved in oxidative resistance and reactive oxygen species metabolism

Gene	Description	Fold change	
		80 μg/mL	800 μg/mL
SCARA3	Scavenger receptor class A, member 3	−1.88	**−5.29**
DUSP1	Dual specificity phosphatase 1	**−2.69**	1.33
AOX1	Aldehyde oxidase 1	−1.89	**−2.65**
VIMP	Selenoprotein S	−1.87	**−2.65**
GSTP1	Glutathione S-transferase pi 1	1.07	**2.12**
HSPA1A	Heat shock 70 kDa protein 1A	**2.13**	1.06
PXDN	Peroxidasin homolog (*Drosophila*)	**2.14**	1.52
GCLM	Glutamate-cysteube ligase, catalytic subunit	1.89	**2.14**
MGST3	Microsomal glutathione S-transferase 3	1.52	**2.16**
TXN	Thioredoxin	1.09	**2.19**
FTH1	Ferritin, heavy polypeptide 1	−1.32	**4.25**
NQO1	NAD(P)H dehydrogenase, quinone 1	**2.13**	**4.28**
HMOX1	Heme oxygenase (decycling) 1	1.84	**4.39**
SRXN	Sulfiredoxin 1	**4.41**	**8.75**
RNF7	Ring finger protein 7	**3.10**	**4.42**
PRDX3	Peroxiredoxin 3	**8.52**	**4.22**
TXNRD1	Thioredoxin reductase 1	**6.13**	**12.36**
PRDX4	Peroxiredoxin 4	**17.55**	**17.50**
PRDX5	Peroxiredoxin 5	**17.69**	**12.60**
OXR1	Oxidation resistance 1	**24.87**	**17.72**

Note: The values above 2.0 or below −2.0 are shown in bold.

each of these genes is directly or indirectly involved in the antioxidative status of the cell.

Promoter analysis in silico

Table 2 presents the transcription factor-binding motifs that are evolutionarily conserved and that showed >50% similarity to the consensus sequence. We identified 16 such transcription factors, nine of which are described in this section.

The promoters of several induced genes included transcription factors of acute myeloid leukemia-1a (AML-1a), which is involved in the anti-inflammatory response,[26] and of the cyclic adenosine monophosphate response element-binding protein (CREB), which is involved in hyaluronic acid synthesis[27] and in regulating development, stress, and inflammatory pathways in fibroblasts.[28]

The *PRDX5*, *PRDX3*, *PRDX1*, and *GCTP1* genes shared the AML-1a transcription factor-binding motif. The *PRDX4*, *HMOX1*, *FTH1*, and *DUSP1* genes shared the CREB transcription factor-binding motif. As an example, Figure S1 shows the CREB identified in the orthologous promoters of the PRDX4 gene in humans, mice, and rats. Based on the motifs shared by the promoters, we created a hypothetical map of the transcriptional regulation using the CellDesigner software (Figure 2).[29]

The promoter of the downregulated DUSP1 gene was tightly occupied by CREBs, including CRE-BP1, CRE-BP1:c-Jun, and CREB:ATF unit motifs. HMOX1, FTH1, and SQSTM1 shared the CREB and NF-E2 transcription factors. SQSTM1 showed FOXO1- and NRF2-binding motifs in the promoter but is not included in Table 1 due to its comparatively low fold increase in expression (1.5 in 800 μg/mL). The EGR1 motif was identified in the promoters of the PRDX5 and TXN genes. EGR1 is a well-known target of the estrogen receptor (ESR1);[30] thus, we conjectured that ESR1 might mediate CES signaling, with consequent activation of the AML-1a and Elk1 transcription factors.

Discussion

This study represents an initial step toward investigating the beneficial effects of chum salmon egg extract on human dermal fibroblast cell cultures. Our results revealed that treatment with CSE extract enhanced the mRNA expression of type I procollagen (COL1A1 and COL1A2) and led to upregulation of peroxiredoxin system components and other antioxidant genes. Phylogenetic footprinting of the affected antioxidant genes predicted 16 transcription factors, including AML-1a and CREB, to be binding promoter regions of genes induced by CSE supplementation.

A previous study has shown the enhancement of type I collagen in human dermal fibroblasts following supplementation with extracts of beluga sturgeon's caviar with added CoQ_{10} and selenium, called LD-1227.[15] They reported a 167% increase in collagen type I mRNA with supplementation

Table 2 Transcription factors (16)

TF name	PSSM	Consensus	MCS	Positive	Control	Gene	Location	Motif
c-Myc:Max	M00322	GCCAYGYGSN	46.83	3[8]	0[0]	*PRDX4*	[46–55]	CCCACGTGGC [0.65] (14)
						PRDX4	[260–269]	CCCACGTGGC [0.65] (14)
						GCLM	[782–791]	GCCGCGCGCT [0.65] (15)
ATF	M00017	CNSTGACGTNNNYC	29.21	2[5]	0[0]	*DUSP1*	[125–136]	GGGTGACGTCAC [0.54] (2)
NF-E2	M00037	TGCTGAGTCAY	23.33	3[4]	0[0]	*FTH1*	[252–262]	TGCTGAGTCAC [0.95] (8)
						HMOX1	[115–125]	TGCTGAGTCAC [0.95] (8)
						SQSTM1	[172–182]	TGCTGAGTCAT [0.95] (13)
						SQSTM1	[19–29]	TGCTGAGTCAC [0.95] (27)
SREBP-1	M00220	NATCACGTGAY	23.33	3[4]	0[0]	*FOXM1*	[62–72]	CGTCACGTGAC [0.77] (3)
						SQSTM1	[440–450]	GACCACCTGAC [0.68] (11)
USF	M00187	GYCACGTGNC	22.59	5[25]	1[1]	*GCLC*	[66–75]	GTCACGTGGC [0.85] (19)
						PRDX4	[46–55]	CCCACGTGGC [0.75] (26)
						PRDX4	[260–269]	CCCACGTGGC [0.75] (26)
						PRDX4	[46–55]	CCCACGTGGC [0.75] (28)
						PRDX4	[260–269]	CCCACGTGGC [0.75] (28)
AP-1	M00517	NNNTGAGTCAKCN	17.46	2[3]	0[0]	*SQSTM1*	[19–31]	TGCTGAGTCACGC [0.54] (21)
						FTH1	[252–264]	TGCTGAGTCACGG [0.54] (28)
CREBATF	M00981	NTGACGTNA	17.46	2[3]	0[0]	*DUSP1*	[127–135]	GTGACGTCA [0.78] (9)
						SIRT2	[437–445]	GTGACGTAG [0.67] (21)
CREB	M00801	CGTCAN	14.37	8[33]	4[4]	*PRDX4*	[571–576]	CGTCAG [0.83] (4)
						DUSP1	[131–136]	CGTCAC [0.83] (10)
						FOXM1	[62–67]	CGTCAC [0.83] (10)
						SIRT2	[1,002–1,007]	CGTCAC [0.83] (10)
						HMOX1	[55–60]	CGTCAT [0.83] (26)
						TXNRD2	[296–301]	CGTCAG [0.83] (26)
						DUSP1	[142–147]	CGTCAC [0.83] (27)
NRSF	M00325	TTYAGCWCCDCGGASAGYRCC	11.58	2[2]	0[0]	*PRDX3*	[71–91]	CTCCGAGCGTCGGTGAGTGGC [0.54] (17)
EGR1	M00243	WTGCGTGGGCGK	11.58	2[2]	0[0]	*PRDX5*	[1,559–1,570]	ATGCGTGGGAGG [0.83] (8)
						TXN	[219–230]	CTGCCTGGGCGG [0.79] (30)
						PRDX5	[2,973–2,982]	ACCGGAAGTG [0.9] (28)
ELK1	M00025	NNNNCCGGAARTNN	11.58	2[2]	0[0]	*PRDX5*	[2,970–2,983]	AGAACCGGAAGTGG [0.54] (18)
NRF2	M00821	NTGCTGAGTCAKN	11.58	2[2]	0[0]	*SQSTM1*	[18–30]	CTGCTGAGTCACG [0.77] (12)
CRE-BP1	M00179	VGTGACGTMACN	11.58	1[2]	0[0]	*DUSP1*	[126–137] +	GGTGACGTCACC [0.82] (4)
						DUSP1	[126–137] –	GGTGACGTCACC [0.82] (4)
CRE-BP1:c-Jun	M00041	TGACGTYA	11.58	1[2]	0[0]	*DUSP1*	[128–135] +	TGACGTCA [0.94] (1)
						DUSP1	[128–135] –	TGACGTCA [0.94] (1)
DEC	M00997	SCCCAMGTGAAGN	11.58	1[2]	0[0]	*PRDX4*	[45–57]	GCCCACGTGGCGG [0.69] (20)
						PRDX4	[259–271]	GCCCACGTGGCGG [0.69] (20)
AML-1a	M00271	TGTGGT	10.29	12[33]	7[20]	*GPX1*	[203–208]	TGCGGT [0.83] (3)

(*Continued*)

Table 2 (*Continued*)

TF name	PSSM	Consensus	MCS	Positive	Control	Gene	Location	Motif
						PRDX3	[6–11] +	TGTGGT [1] (3)
						PRDX3	[6–11] –	TGTGGT [1] (3)
						GPX1	[692–697]	TGTGGT [1] (3)
						PRDX5	[153–158]	TGTGGT [1] (8)
						PRDX3	[43–48]	TGTGGT [1] (9)
						GCLC	[281–286]	TGTGGT [1] (11)
						TXNRD1	[427–432]	TGTGGT [1] (18)
						PRDX1	[49–54]	TGTGGT [1] (19)
						GCLC	[128–133]	TGTGGT [1] (20)
						GSTP1	[15–20]	TGTGGT [1] (23)
						PRDX3	[18–23]	TGTGGT [1] (24)
						GSTP1	[28–33]	TGTGGT [1] (25)

Notes: Columns are as follows: PSSM, matrix number in public Transfac database; consensus, motif consensus using International Union of Pure and Applied Chemistry (IUPAC) nucleotide ambiguity codes (http://www.bioinformatics.org/sms/iupac.html); positive, unique number of genes (out of the 33 induced genes) with the respective motif and the number of motif copies in brackets; control, unique number of genes (out of the 31 uninduced genes) with the respective motif and the number of motif copies in brackets; gene, selected genes from the positive set; location, location in the promoter toward the transcriptional start site, with a +/– indication when the motif is on the reverse or complement strand; motif, motif sequence identified, matching score to the consensus in square brackets, and the number of the Pareto front in parentheses.

Abbreviations: AML-1a, acute myeloid leukemia-1a; CREB, cyclic adenosine monophosphate response element-binding protein; MCS, motif conservation score; TF, transcription factor.

relative to control. Similarly, our present results showed that CSE supplementation led to COL1A1 increases of 211% with the higher dose and 125% with the lower dose. Although comparison between the previous study and our present study must be made cautiously, we can safely state that our present findings add to the evidence that marine organism-derived extracts can promote antiaging activity, ie, repair of extracellular matrix degeneration from the aging process.

RT^2 Profiler PCR Array analysis revealed the strongest upregulation for the *PRDX4*, *PRDX5*, and *OXR1* genes, with expressions increased by >10-fold with both high and low doses of CSEs. The mechanism of OXR1 activity has not yet been determined, but it plays an important role in protection against oxidative damage.[31] Peroxiredoxin family genes along with *TXN*, *TXNRD1*, and sulfiredoxin (*SRXN1*) constitute the peroxiredoxin system, in which peroxiredoxin reduces hydrogen peroxide and TXN acts as an electron donor to peroxiredoxins.[32] Most of the genes of the peroxiredoxin system were prominently upregulated by CSE supplementation, and hence we can infer the interplay of the peroxiredoxin system for antioxidant protection. PRDX3 localizes to mitochondria, PRDX4 is found in endoplasmic reticulum, and PRDX5 is mainly present in peroxisomes, mitochondria, and cytosol.[33] PRDX4 activity covers a broad spectrum, showing involvement in antioxidant activity in leukemia with the AML-1 and NF-κB genes,[34] as well as in controlling neurogenesis in normal cells.[35] NF-κB is involved in the oxidative stress response[36] and is reportedly specific for the total oxidative stress pathway but not for the CSE-stimulated gene subset. The GPX system is a major pathway for hydrogen peroxide elimination, similar to the peroxiredoxin system and catalase. It is unclear why GPXs did not show prominent fold changes despite prominent upregulation of several genes involved in the GPX system (MGST3, GCLM, and GSTP1). Conceivably, our result might offer insights to dissect the overlapping roles of the GPX and peroxiredoxin systems. Several genes involved in oxidative resistance, such as *GPX1*, *SCARA3*, *DUSP1*, and *AOX1*, showed reduced expressions with both CSE concentrations and some with a fold change of below –2. However, the number of prominently downregulated genes was 2 with 80 µg/mL and 3 with 800 µg/mL conditions, while the number of prominently upregulated genes was 10 with 80 µg/mL and 14 with 800 µg/mL conditions. With both CSE concentrations, more genes were highly upregulated than were highly downregulated. On the basis of these results, it is reasonable that we infer that CSE supplementation improved the overall antioxidant activity of the cells, with this effect seeming to be dose dependent.

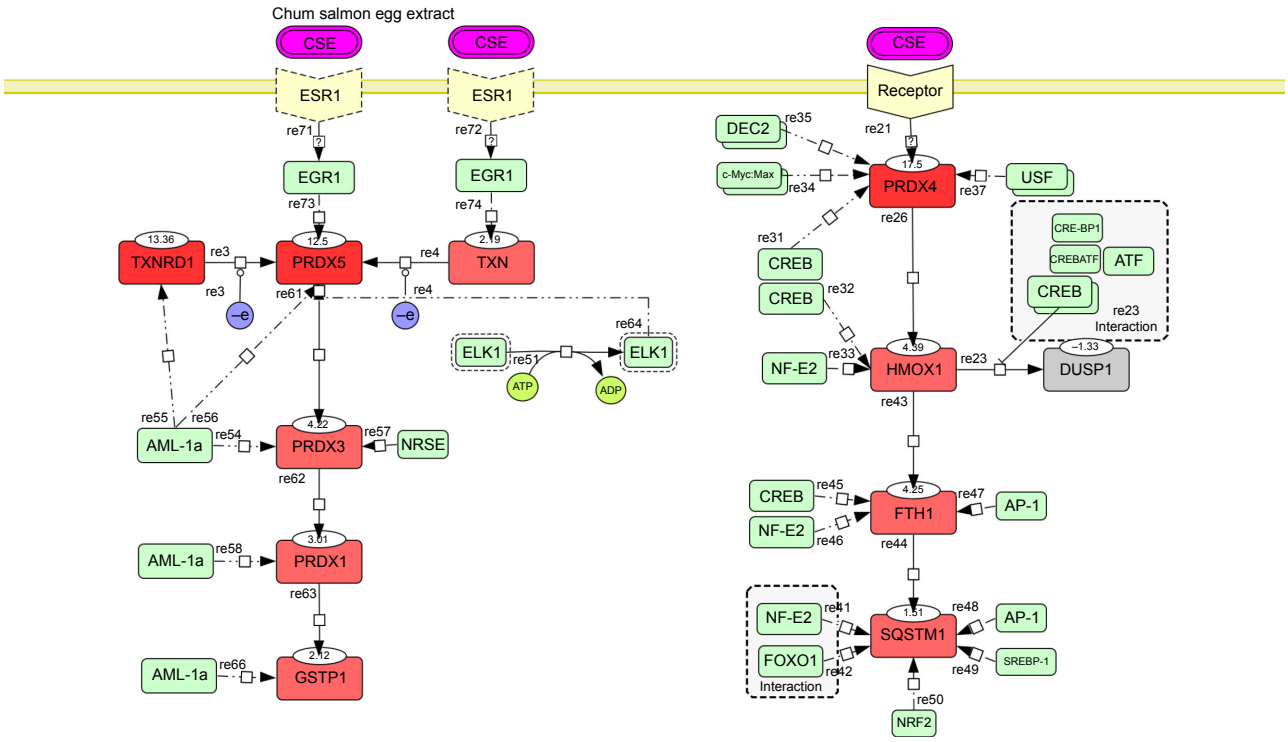

Figure 2 Hypothetical map of transcription regulation.
Notes: Red rectangles represent differentially regulated genes with the fold change indicated at the top edge. Solid arrows show the proposed gene activation cascade based on the transcription factor motifs shared by the genes, the activation levels, and available data from the literature. Green rectangles represent transcription factors whose sites are evolutionarily conserved among three species, with dashed arrows pointing to the associated genes. Pairs of overlapping green rectangles indicate that there were two copies of the identified motif. Dashed rectangles enclose interacting transcription factors. Hypothetical receptor genes are shown in yellow.
Abbreviation: CSEs, chum salmon eggs.

In our dataset, we identified two main kinds of transcription factors: inflammation-response transcription factors (AML-1a, EGR1, and ELK1)[37] and DNA damage repair and antioxidant activity transcription factors (CREB, NF-E2, NRF2, c-Myc:Max,[38] USF,[39] and FOXO1). The involvement of the oncogene AML-1a was surprising. On the other hand, CREB is well known for its role in limiting ROS toxicity in the brain,[40,41] as well as its involvement as an effector-like nuclear protein in stress and inflammatory response pathways.[28] Together with DUSP1, CREB elicits negative regulation of cellular proliferation under oxidative stress. FOXO1 is a longevity transcription factor[42] that promotes repair of DNA oxidative damage, together with the NF-E2 transcription factor[43] whose motifs were presently identified in the promoters of the HMOX1, FTH1, and SQSTM1 genes. NRF2, whose motif was found in the SQSTM1 promoter region, is well known for its involvement in oxidative stress responses[44] and photoaging resistance.[45] EGR1 induces cell apoptosis upon UV irradiation stress,[46] and its involvement presupposes an involvement of estrogen receptor (ESR1) in CSE-induced signaling, as shown in Figure 2.

Although it is difficult to predict precisely what triggers the observed beneficial modulations involving numerous genes and transcription factors, we can hypothesize several plausible responsible molecules. Table 3 describes the nourishing qualities of chum salmon egg. One candidate molecule is astaxanthin, which is a precursor of vitamin A17 and an effective antioxidant. Due to its beneficial properties, astaxanthin is already included in some cosmeceutical products, for example, Fujifilm Astalift lineup (http://www.astalift.com.sg). Other candidate active components are yolk-specific proteins, such as phosvitin and lipovitellin. Phosvitin is one of the most phosphorylated proteins in nature and possesses a strong metal-binding ability that makes it useful in antioxidant defense[47] and immunoprotection.[48] Lipovitellin is classified as a glycolipoprotein and is involved in the

Table 3 The composition of chum salmon egg

Content	Concentration (per 100 g)	Examples
Fatty acids	14.0 g	Oleic acid (ω9), linoleic acid (ω6), palmitoleic acid (ω7), and DHA and EPA (ω3)
Proteins	31.2 g	Phosvitin, lipovitellin, and lysozyme
Vitamin E	7.2 mg	
Vitamin A	374 μg	
Astaxanthin	0.99 mg	

Abbreviations: DHA, Docosahexaenoic acid; EPS, eicosapentaenoic acid.

storage of nutrients for growth as well as the immune defenses of embryos and larva.[49] Moreover, vitellogenin, the precursor of phosvitin and vitellin, is broadly conserved among oviparous animals and is synthesized in the liver and incorporated into oocytes under the control of estrogen.[50] Both environmental and injected estrogens influence vitellogenin expression and blood concentration.[51,52] Thus, the presence of vitellogenin in CSEs might induce EGR1 upregulation, prompting further potential positive effects on skin rejuvenation[53] and skin esthetics. There remains a need for further investigations of estrogen receptors in relation to skin aging.

Conclusion

In conclusion, our present findings suggested the possibility that CSEs could be effectively used as a source of cosmeceutically active ingredients that exert antiaging and antioxidative effects on the skin. We have also hypothesized the pathway connecting stimulation with CSE extract with the upregulation of several antioxidant genes involving transcription factors, which is characterized by certain beneficial effects to the cell.

Disclosure

The authors report no conflicts of interest in this work.

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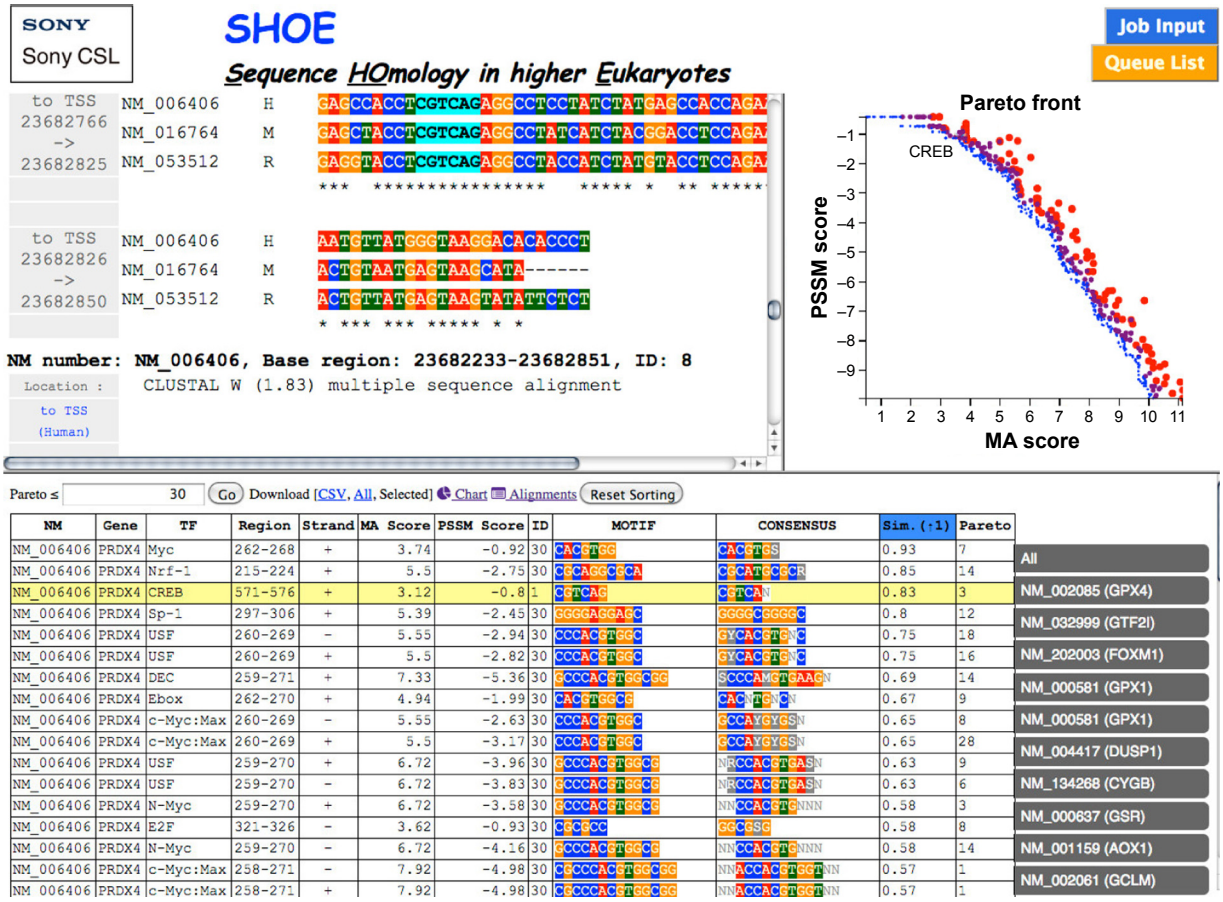
Supplementary material



NM	Gene	TF	Region	Strand	MA Score	PSSM Score	ID	MOTIF	CONSENSUS	Sim. (↑1)	Pareto
NM_006406	PRDX4	Myc	262-268	+	3.74	-0.92	30	CACGTGG	CACGTGS	0.93	7
NM_006406	PRDX4	Nrf-1	215-224	+	5.5	-2.75	30	GGCAGGCGCA	CGCATGCGCR	0.85	14
NM_006406	PRDX4	CREB	571-576	+	3.12	-0.8	1	CGTCAC	CGTCAN	0.83	3
NM_006406	PRDX4	Sp-1	297-306	+	5.39	-2.45	30	GGGGAGGAGC	GGGGCGGGGC	0.8	12
NM_006406	PRDX4	USF	260-269	-	5.55	-2.94	30	CCCACGTGGC	GYCACGTGNC	0.75	18
NM_006406	PRDX4	USF	260-269	+	5.5	-2.82	30	CCCACGTGGC	GYCACGTGNC	0.75	16
NM_006406	PRDX4	DEC	259-271	+	7.33	-5.36	30	GCCCACGTGGCGG	SCCCAMGTGAAGN	0.69	14
NM_006406	PRDX4	Ebox	262-270	+	4.94	-1.99	30	CACGTGGCG	CACGTGNCN	0.67	9
NM_006406	PRDX4	c-Myc:Max	260-269	-	5.55	-2.63	30	CCCACGTGGC	GCCAYGYGSN	0.65	8
NM_006406	PRDX4	c-Myc:Max	260-269	+	5.5	-3.17	30	CCCACGTGGC	GCCAYGYGSN	0.65	28
NM_006406	PRDX4	USF	259-270	+	6.72	-3.96	30	GCCCACGTGGCG	NRCCACGTGASN	0.63	9
NM_006406	PRDX4	USF	259-270	-	6.72	-3.83	30	GCCCACGTGGCG	NRCCACGTGASN	0.63	6
NM_006406	PRDX4	N-Myc	259-270	+	6.72	-3.58	30	GCCCACGTGGCG	NNCCACGTGNNN	0.58	3
NM_006406	PRDX4	E2F	321-326	-	3.62	-0.93	30	GGCGCC	GGCGSG	0.58	8
NM_006406	PRDX4	N-Myc	259-270	-	6.72	-4.16	30	GCCCACGTGGCG	NNCCACGTGNNN	0.58	14
NM_006406	PRDX4	c-Myc:Max	258-271	-	7.92	-4.98	30	GGCCCACGTGGCGG	NNACCACGTGGTNN	0.57	1
NM_006406	PRDX4	c-Myc:Max	258-271	+	7.92	-4.98	30	GGCCCACGTGGCGG	NNACCACGTGGTNN	0.57	1

Figure S1 Visualization of the results of promoter analysis of the PRDX4 gene, especially the CREB transcription factor-binding motif.
Notes: The upper left part of the figure shows the orthologous species alignment. The upper right panel shows the Pareto front graph with the CREB motif allocated on the first Pareto front, indicating its strong cross-species conservation and strong matching with a known matrix. The table below lists all candidate motifs with their similarity matching score to the consensus motif and their Pareto front number.
Abbreviation: CREB, cyclic adenosine monophosphate response element-binding protein.