

Human Skin Gene Expression, Attributes of Botanicals: *Angelica sinensis*, a Soy Extract, Equol and its Isomers and Resveratrol

Edwin D. Lephart*

Department of Physiology and Developmental Biology and The Neuroscience Center, Brigham Young University, Provo, Utah, USA

Abstract

Because of its accessibility, skin was one of the first organs to be examined by gene technologies. Microarray/mRNA techniques have demonstrated the valuable aspects of this methodology for the elucidation of and the quantification for changes in human skin related-genes. It is important to review/understand how botanicals influence human skin gene expression (by stimulation or inhibition of certain genes) and to compare these biomarkers to the known mechanisms of skin aging. This review covers how human skin genes are modulated by 1) enhanced wound healing with an extract of a well-known medicinal plant in Asia, *Angelica sinensis*, 2) UV sunlight exposure that represents the main cause of photoaging or extrinsic skin aging and subsequent protection by a soy extract, 3) equol and their isomers that stimulate collagen and elastin while at the same time inhibit aging and inflammatory biomarkers and 4) resveratrol, the most high profile phytochemical known by the general public that displays some properties similar to equol with the additional benefit of stimulating the anti-aging surtuin or SIRT1 biomarker. Thus, the protective influences of botanicals/phytochemicals elucidated herein provide potential applications to improve human skin health.

Keywords: Botanicals; Phytochemicals; Polyphenols; Human skin; Gene expression; UV irradiation; Equol; Resveratrol

Abbreviations: AP1: Activator Protein 1; DMSO: Dimethyl Sulfoxide; ECM: Extracellular Matrix; EFT: Epidermal Full Thickness; ERR: Estrogen Related Receptor; HDFC: Human Dermal Fibroblast Cultures; Nrf2: Nuclear-Factor-Erythroid 2-Related Factor; qPCR: Quantitative Polymerase Chain Reaction; ROS Reactive Oxygen Species; SAM: Selective Androgen Modulator; SIRT: Sirtuin Activator; UV: Ultra Violet; 5 α -DHT: 5 α -Dihydrotestosterone

Introduction

This section outlines the scope for this review that covers: 1) a brief background on human skin characteristics, 2) a short historical description of how botanicals have been used for medicinal purposes, 3) a sketch on the advancements in scientific technology of dermal biology via gene expression, and 4) the different phytochemical materials tested as extracts or single compounds that display changes on human skin gene expression via biomarker endpoints using similar methodologies. For each published report described, along with supportive references, brief notations to human skin components relating to structure/function mechanisms are mentioned to highlight the dynamics of anti-aging and tissue-repair.

Because of its accessibility, skin was one of the first organs to be examined by microarray technologies [1]. It is well established that a gradual decline in overall skin physiology occurs with aging. This includes structural changes in the epidermis (keratinocytes) and dermis along with functional changes in fibroblasts and other cellular components that are linked to the expression of skin-related genes and proteins [2]. For example, dermal fibroblasts contribute to the production of many extracellular matrix proteins (ECM) such as collagen, elastin, elastase, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs) and other molecules such as anti-oxidants and anti-aging factors that are important for maintaining healthy skin [2-4]. Targeting skin aging to achieve a youthful appearance has a long history including home/traditional plant remedies for wound healing, anti-inflammatory and topical analgesia [5], but more recent technology-based active ingredients utilizing plant extracts are now common based on gene array studies [1].

In this regard, botanicals have been used as medicine for thousands of years with one written record on Egyptian papyrus describing

medicinal plant usage dating back to 1500 B.C. [6,7], while other references suggest medicinal plants date back to at least 5000 years to the Sumerians [5]. Botanicals have not only been used as medicine, but their incorporation within the last 10-20 years into cosmeceuticals has dramatically increased. For example, botanicals represent one of the largest proportion of active ingredients in the cosmeceutical market where they function as skin protectants, whitening, anti-wrinkle, and anti-aging agents [8-12].

In skin aging, many events lead to the production of endogenous reactive oxygen species (ROS) that in turn increase the production of matrix metalloproteinases (MMPs) that then breakdown the important collagen dermal and elastin fibers [13]. These insults can lead to dermal fibroblast collapse that in turn speed up the process of intrinsic aging with the generation of wrinkles [13] (Figure 1).

For all individuals the aging mechanisms are continually in play, and the dermatological focus must be on decreasing or slowing down the aging of the skin by increasing collagen and elastin components, while at the same time decreasing the production of MMPs (that breakdown collagen and elastin) [14].

Especially with the advancements in technology and the exchange of scientific information, where physicians and patients have a greater awareness of dermal biology, several scientific techniques have been developed. Traditional assays include cell and/or organotypic cultures to investigate the influence of a compound on human skin. Conversely, genetic testing strategies (gene array/mRNA levels) to identify and quantify gene expression are relatively new [1].

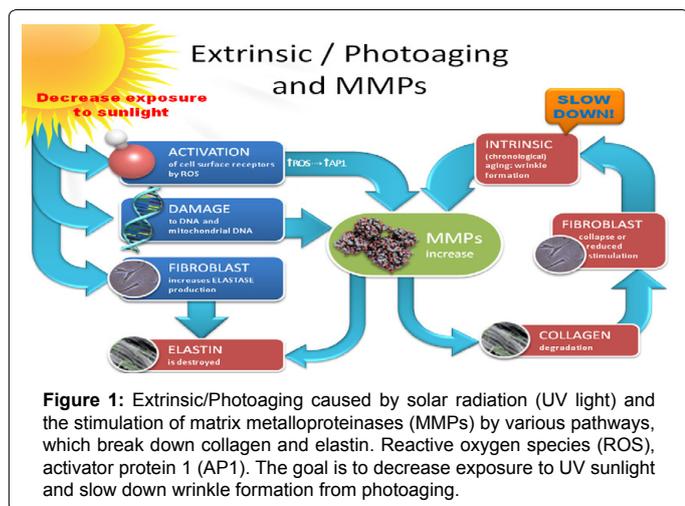
It is important to understand how botanicals (and other compounds)

***Corresponding author:** Edwin D. Lephart, Department of Physiology and Developmental Biology and The Neuroscience Center, Brigham Young University, Provo, Utah, USA, Tel: 801-422-2006; E-mail: Edwin_Lephart@byu.edu

Received February 11, 2015; **Accepted** March 30, 2015; **Published** April 07, 2015

Citation: Lephart ED (2015) Human Skin Gene Expression, Attributes of Botanicals: *Angelica sinensis*, a Soy Extract, Equol and its Isomers and Resveratrol. Gene Technology 4: 119. doi: [10.4172/2329-6682.1000119](https://doi.org/10.4172/2329-6682.1000119)

Copyright: © 2015 Lephart ED. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



Gene	Modulation	Function
ExtracellularMatrix Proteins		
CLDN1	200	Produces Claudin 1, tight junctions, establish barrier control/flow of molecules betweenepithelium
CLDN4	200	Produces Claudin 4, same as CLDN1
COL16A1	320	Collagen type-XVI, fibril-forming, tensile strength in the skin/dermal integrity
COL17A1	420	Collagen type-XVII, component of hemidesmosome, anchoring epithelial cells to basement membrane
LAMC2	390	Laminin C2, produces laminin γ-2, key component basement membrane (basal lamina)
LAMA3	260	Laminin A3, produces laminin 5, cell adhesion, differentiation of keratinocytes/anchoring filaments
Proteinases of collagen		
ADAM9	-170	Produces Adam9, disintegrin/metalloproteinase (interacts with collagen type I) delays wound healing
Tissue Repair		
HAS3	170	Hyaluronan synthase 3, hyaluronic acid, wound healing/tissue repair (lubrication)
HBEGF	160	Heparin-binding epidermal growth factor, wound healing/regenerative process of skin
Antioxidants		
OD2	170	Superoxide dismutase 2, anti-oxidant activity of oxygen and free radicals associated with tissue injury

Table 1: Wound-relevant Skin Genes Modulated by *Angelica sinensis* (SBD.4) in Epidermal full thickness cultures stimulation or inhibition (-) expressed vs. controls (by percentages).

positively influence human skin gene expression (by stimulation or inhibition of certain genes) and compare these biomarkers to the known mechanisms of skin aging. Thus, the goal is to increase the gap between chronological and biological aging where ECM constituents of the dermis and epidermal components are enhanced and biologically active with aging.

Surprisingly, there are few published studies that have examined botanicals (even high-profile phytochemicals, e.g., resveratrol) for their attributes on human skin gene expression. Additionally, the published studies are not uniform in their experimental design. For example, one study took the approach of examining an aqueous isolate of a medicinal herb that contained various earth metals, another investigated UV exposure along with a soy extract, while other studies took the approach of testing single polyphenolic molecules in gene expression analysis via specific biomarkers of human skin-related genes. All of the studies used

human skin biopsies and/or epidermal full thickness (EFT) cultures (from human biopsies) representing human skin barrier equivalents. Having these comparative, but mixed approaches in hand, this review covers the following botanicals that are in medicinal plants: a) *Angelica sinensis*, c) UV exposure plus a soy extract, d) equol (isomers) and e) resveratrol. A brief descriptive background, followed by how each test material was derived (if applicable) along with the human skin gene analysis from each study is presented.

Angelica sinensis

Enhanced wound healing by an extract of a medicinal plant in Asia.

Angelica sinensis is a well-known medicinal plant in Asia used to treat menstrual cycle irregularities and angina pectoris [15-17]. Zhoa et al., studied a subset of the *Angelica sinensis*' active ingredients by isolating from the roots of this medicinal plant via an aqueous extraction with subsequent fractionation [18]. This isolate (which the authors named SBD.4) was previously shown to stimulate wound healing in genetically diabetic mice and in human skin grafts on mice [19]. The elemental composition of the SBD.4 contained high concentrations of phosphorus (5.3 g/kg), calcium (3.8 g/kg) and magnesium (2.8 g/kg) along with low levels of heavy metals. In order to determine the influence of the SBD.4 isolate on wound-relevant skin genes, Zhoa et al., used EFT tissue cultures incubated in the absence or presence of SBD.4 at 1 mg/ml for 48 hours [19]. In this study, the tissues were processed via microarray analysis and mRNA levels were quantified using vehicle controls as the baseline. The data were expressed as the percentage of controls for stimulation or inhibition for each sample tested [19].

As summarized in Table 1, the microarray results by Zhoa et al., demonstrated the SBD.4 isolate had positively influenced wound-relevant genes [19]. For example, it was observed that the extracellular matrix genes significantly increased ranging from 170 to 420 percent for the claudins, collagens and laminins, important proteins for structural anchoring or connectivity along with increasing the tensile strength of various skin components. Also, the anti-oxidant, superoxide dismutase significantly increased by 170 percent along with the tissue repair genes, hyaluronan synthase and heparin-binding epidermal growth factor (ranging from 160 to 170 percent). Conversely the gene that encodes the enzyme that breaks down collagen, i.e., ADAM9 was significantly inhibited by 170 percent, which is known to delay wound healing (Table 1) [19].

Human Epidermal Full Thickness Cultures were incubated in the absence or presence of SBD.4 (1 mg/ml) for 48 hours, after which wound-relevant skin genes were quantified by gene array/mRNA analysis [19] (doi:10.1684/ejd.2011.1599). Presumably n=4 to 6 samples were assayed per biomarker in this study, results expressed as mean values; <http://bmbolstad.com/stuff/qnorm.pdf> using Genesifter software. mRNA levels were quantified using vehicle controls as the baseline and all data were expressed as the percentage of controls for stimulation or inhibition for each sample tested*. The SBD.4 treatment was defined as the aqueous isolate of the Chinese medicinal herb, *Angelica sinensis*, containing heavy metals that previously demonstrated wound healing properties in genetically diabetic mice and human skin grafts [18].

Thus, the aqueous isolate, SBD.4 was shown to have wound healing properties based upon the molecular expression of various skin-related genes that would enhance skin tissue repair. It is interesting to note that the high concentration of earth metals in SBD.4 may play some positive role in the expression of the wound-relevant genes revealed from this study by Zhoa et al., [19].

Extrinsic (photo) aging of human skin and the protective attributes of a soy extract

The study by Sudel et al., examined the changes in skin-related genes from human dermal biopsies exposed to specific ultraviolet (UV) dosing in younger versus older subjects [20]. It is known that extrinsic or photo aging such as UV exposure from sunlight accounts for 90 percent of overall skin aging [13]. Among the skin changes with UV exposure are reductions in different types of collagen and, increases in the matrix metalloproteinases (MMPs) that breakdown collagen and elastin (Figure 1) [13]. To confirm the in vivo dermal biopsies results reported by Sudel et al., in the same study, the authors used primary human dermal fibroblast cultures (HDFC) that were exposed to UV irradiation for up to 48 hours from older adult donors.

The observed results of these experiments are summarized in Table 2 [20]. Collagens 1A1, 1A2, 3A1, 6A1, 6A2, 16A1 and 18A1 and hyaluronan synthase (HAS3) were significantly reduced 24 hours after UV exposure in both young (18-35 years of age) and old volunteers (57-70 years of age) (Table 2) [20].

In vivo results: from volunteers irradiated with two individual doses with a Solar Sun Stimulator for minimal erythematous effects. Skin biopsies were taken 24 hours after irradiation by age groups. In vitro results: from primary human dermal fibroblast cultures (HDFC) exposed to UV irradiation after 48 hours. Skin-related gene expression was quantified by gene array/mRNA analysis from the in vivo and in

vitro conditions (mean values), NA=not assayed. Interestingly, when a 2% Soy Extract (in an oil-water emulsion) was tested (10 µg/ml) in HDFC this significantly increased HAS by 350% and COL1A1 by 160% vs. control (baseline levels) in the in vitro cultures (protein data not shown). Data displayed from references [20,21].

Additionally, from another study, by Sudel et al., using similar experimental conditions the MMPs were significantly increased [21]. To validate the in vivo results in vitro studies used HDFC exposed to UV irradiation for 48 hours were performed in samples from subjects 57-61 years of age by Sudel et al. [20].

In comparing the observed human skin gene expression changes with UV exposure in aged skin versus younger skin the results were remarkably similar as to the direction and the magnitude of the response. Also, the in vitro results were similar to the in vivo data, confirming the significant reductions of various types of collagen and hyaluronan synthase along with the significant elevation of MMP-1 and MMP-3 (Table 2) [20]. Finally, to test whether a soy extract may have skin protecting properties, Sudel et al., used a 2% Soy Extract (in an oil-water emulsion) that was assayed (10 µg/ml) in HDFC significantly increased HAS3 by 350% and COL1A1 by 160% versus control (baseline levels) in the in vitro cultures (data not shown graphically) [20].

Thus, this study by Sudel et al., confirmed the molecular changes in skin gene expression of human samples exposed to UV irradiation from young and old volunteers using in vivo and in vitro methodologies where several collagen types were inhibited and the enzymes (MMPs) that breakdown collagen and elastin were stimulated. Finally, the skin enhancing properties of a soy extract suggested beneficial influences on extracellular matrix genes to protect and improve human skin health.

Human skin-related genes modulated by: R-equol vs. Racemic equol vs. S-equol

As highlighted by the reports by Setchell and Clerici, Lund et al., and Blake et al., equol has recently caught the interest of researchers because of its powerful antioxidant activity and its unique molecular and biochemical messenger properties with implications in treating age-related diseases [22-24]. Of particular interest, equol has an affinity for estrogen receptor beta, which is abundant in keratinocytes of the epidermis and fibroblasts in the dermis, as reported by several investigators [14,25-27]. Equol is also a selective androgen modulator (SAM), having the ability to specifically bind 5α-dihydrotestosterone (5α-DHT) and inhibit its potent negative actions in skin [14,25]. Finally, Hirvonen et al., reported that equol has the ability to bind to estrogen related receptor (ERR) gamma, which has important implications for anti-aging in human skin [25,28,29].

Equol is an isoflavonoid or polyphenolic molecule derived from daidzein that is a natural phytochemical present in soy beans and other plants. Also, recent published reports have demonstrated that equol is present in plant products such as beans, cabbage, lettuces, tofu and other food products [30-33]. Equol can be found as R- and S-configuration or isomers [34-36] and Setchell et al., has reported the metabolism of R- and S-equol in humans which appears to be similar [34,36]. Therefore, humans are exposed to this polyphenolic compound from different plant and food sources regardless of age, gender or geographical location with scientific data to support a consumption/exposure record that appears to be safe [35,37,38]. Moreover, Beyer et al., have reported that equol is known to increase nuclear-factor-erythroid 2-related factor 2 (Nrf2) that plays a key role in the cellular defense against oxidative and xenobiotic stressors by its capacity to induce the expression of genes which encode detoxifying enzymes and

Gene				Function
Extracellular matrix proteins				
COL1A1	-29	-47	-38	Collagen type-I, major (most abundant) structural protein in skin
COL1A2	-38	-51	-52	Collagen type-II, fibrillar protein in most connective tissue
COL3A1	-26	-41	-44	Collagen type-III, fibrillar protein abundant in youth, associated with COL1A1
COL5A1	NA	NA	-57	Collagen type-V, encodes α-chain, a lower abundant fibrillar protein
COL6A1	-47	-51	-51	Collagen type-VIA1, major structural protein in micro fibrils
COL6A2	-42	-46	-41	Collagen type-VIA2, important binding/organizing extracellular matrix
COL16A1	-41	-28	-56	Collagen type-XVI, fibril-forming, tensile strength in the skin/dermal integrity
COL18A1	-49	-40	NA	Collagen type-XVIII, produces endostatin-inhibits angiogenesis/tumor formation
Proteinases of collagen/Elastin				
MMP-1	22,660	35,700	4,430	Matrix Metalloproteinase-1, breaks down interstitial collagens types I, II, and III
MMP-3	29,350	38,220	2,550	Matrix Metalloproteinase-3, breaks down collagens/elastin and ↑ other MMPs
Growth Factor				
CTGF	NA	NA	-44	Connective Tissue Growth Factor, tissue wound repair
Tissue Repair				
HAS2	NA	NA	202	Hyaluronan synthase 2, hyaluronic acid, wound healing/tissue repair (lubrication)
HAS3	221	333	NA	Hyaluronan synthase 3, same as HAS2,
HSP-47	NA	NA	-45	Heat Shock Protein-47, chaperon to collagens (I, II, III, IV and V)

Modulation: Stimulation or Inhibition (-) expressed vs. controls (by percentages).

In Vivo	In Vivo	In Vitro
18-35 years	57-70 years	57-61 years
n=9	n=10	n=10

Table 2: Human skin UV exposure: Alterations in skin-related gene expression and benefits of a Soy-extract.

antioxidant proteins in skin [39,40]. Finally, as reported by Setchell and Clerici, equol research has dramatically increased within the past decade [22] and this polyphenolic molecule along with other botanical compounds have wide use in personal care products such as cosmeceutical ingredients.

Since equol can be expressed as isomers, a recent study described a comprehensive investigation on equol as: R-equol, racemic equol or S-equol to determine their differential expression of skin-related genes and determine the percutaneous absorption in human skin using Franz cell techniques [25].

From this recent report, from the qPCR/mRNA studies quantifying genes expression, only three genes displayed the greatest significant expression by S-equol, whereas 17 genes displayed the greatest significant levels (either stimulation or inhibition) by R-equol and/or racemic equol, such as extracellular matrix proteins (i.e., collagen and elastin, etc.), nerve growth factor, aging genes [FOS, 100 A8 and 100 A9 calcium-binding proteins, 5 α -reductase type 1, and matrix metalloproteinases (MMPs 1, 3 and 9), and inflammatory genes (e.g., interleukin-1 alpha, interleukin-6, and cyclooxygenase-1) [14,25]. These data from Gopaul et al., and Lephart (in general) are summarized in Table 3 according to gene categories.

In summary for the investigations of the isomers of equol, the results illustrated the significant differences in mirror-image molecules of equol for skin-related gene expression and clearly demonstrated that R-equol and/or racemic equol are better molecules for dermal gene expression in anti-aging effects compared to S-equol (Table 3) [25].

Each Gene Symbol/Name is color-coded indicating the equol form with the greatest (stimulation or inhibition) expression level.

Resveratrol's attributes in modulating human skin-related gene expression

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a naturally occurring polyphenolic compound found in red grapes (wine) and many plants [41-44]. Since the discovery of its chemopreventive activity from John Pezzuto's laboratory [44] it is the most high-profile, natural molecule known to the public with many reported health benefits. Several investigators have described the uses for resveratrol that include anti-carcinogenic, anti-inflammatory, cardioprotective, and neuroprotective properties, with continuing research which has uncovered additional potential uses for this compound such as, sirtuin activator (SIRT) or an anti-aging, anti-obesity and anti-diabetic agent [45-51]. Baumann along with Evans and Johnson have reported on the role of phytonutrients in skin health that have shown promise as photo-protectants against sun exposure, antioxidants, and these natural molecules display many other beneficial actions [52,53].

While resveratrol has been studied in many different applications including treating human skin, it is surprising that this polyphenolic molecule had not been examined directly in a comprehensive manner quantifying the expression of human gene biomarkers such as the extracellular matrix proteins, like collagen, elastin and other important human dermal components. Thus, a recent study examined the influence of resveratrol on the expression of skin genes by quantifying various biomarkers using human dermal models to determine whether resveratrol may benefit skin health by its anti-aging properties.

Gene	Gene Name	R-Equol	Racemic equol	S-equol	Function
Extracellular matrix proteins					
COL1A1	Collagen type-I	210 + 11	235 + 17>	185 + 16	(most abundant) proteins in skin
ELN	Elastin	175 + 19>	175 + 19>	1 + 1	elastic/bounce-back properties in skin
TIMP1	Tissue Inhibitor of Matrix Metalloproteinase 1	2 + 2	200 + 15<	150 + 9<	inhibitor of MMPs
Proteinases of collagen/elastin					
MMP1	Matrix Metalloproteinase 1	-890 + 25>>	-535 + 80>	-325 + 23	breaks down collagens I, II, and III
MMP3	Matrix Metalloproteinase 3	-885 + 29>>	-795 + 38>	-330 + 45	breaks down collagens and elastin
MMP9	Matrix Metalloproteinase 9	-1,375 + 106>>	-1,080 + 60>	-710 + 80	↑ angiogenesis and IL-1B, remodels extracellular matrix
Antioxidant					
MT1H	Metallothionein-1H	2,100 + 70	2,270 + 63<	3,840 + 37<<	binds various heavy metals
MT2H	Metallothionein-2H	NA	510 + 34	NA	binds various heavy metals
PCNA	Proliferating Cell Nuclear Antigen	235 + 15	285 + 11	325 + 10	DNA synthesis and repair/tissue repair
SOD	Superoxide dismutase 2	NA	200 + 3	NA	anti-oxidant activity- of oxygen and free radicals
TXNRD1	Thioredoxin reductase 1	NA	215 + 18	NA	anti-oxidant, anti-apoptotic
Growth Factor					
NGF	Nerve Growth Factor	3,350 + 15>>	2,860 + 32>	1,620 + 22	skin tissue repair, cell survival
Aging					
FOS	FOS (Immediate early gene)	320 + 19	340 + 9>	315 + 9	protection against photoaging
S100 A8	S100 Calcium-binding Protein A8	-2,050 + 24♣	-2,200 + 32♦♦	-580 + 28	increases with age, proinflammatory.
S100 A9	S100 Calcium-binding Protein A9	-1,850 + 17♣	-2,250 + 27♦♦	-525 + 3	same as S100 A8
SRD5A1	Steroid 5 α -Reductase 1	-200 + 9♣♣	-175 + 10♦	0	androgen production of 5 α -DHT
Inflammatory					
IL1A	Interleukin 1 Alpha	-1,385 + 17♣	-1,700 + 27♦♦	-990 + 42	pro-inflammatory
IL1R2	Interleukin 1 Receptor 2	-1,730 + 26	-2,250 + 31♦♦	-1,675 + 4 5	pro-inflammatory
IL6	Interleukin 6	-550 + 20♣♣	-455 + 39♣	-375 + 29	pro-inflammatory
IL8	Interleukin 8	-295 + 21	-345 + 24	-445 + 33♣	pro-inflammatory
PTGS1 (COX1)	Cyclooxygenase 1	-360 + 10♣♣	-265 + 25	-200 + 15	pro-inflammatory, prostaglandins
TNFRSF1A	Tumor Necrosis Factor Receptor 1A	-310 + 10♣♣	-250 + 19	-205 + 22	pro-inflammatory

Table 3: Human Skin-related Genes Modulated by: R-Equol vs. Racemic Equol vs. S-Equol (@1.2%) in EpiDermal Full Thickness Cultures.

Gene	Gene name	Modulation	Role
Anti-Aging			
SIRT 1	silent mating type information regulator	180 + 10	Sirtuin, anti-aging, de-acetylates proteins and inhibits MMPs
Extracellular matrix proteins			
COL1A1	Collagen type-I	225 + 25	(most abundant) structural protein in skin
COL3A1	Collagen type-III	230 + 12	fibrillar protein abundant in youth, associated with COL1A1
COL1A1	Collagen type-IV	160 + 25	separates/supports basement membranes
ELN	Elastin	180 + 11	protein fiber, elastic/bounce-back properties in skin
LOX	Lysyl Oxidase	180 + 5	cross links collagen and elastin fibers
TIMP1	Tissue Inhibitor of Matrix Metalloproteinase 1	220 + 10	inhibitor of MMPs
Proteinases of collagen/elastin			
MMP1	Matrix Metalloproteinase 1	-180 + 11	breaks down collagens I, II, and III
MMP9	Matrix Metalloproteinase 9	-490 + 22	↑ angiogenesis and IL-1B, remodels ECM
Antioxidant			
CAT	Catalase	180 + 9	anti-oxidant enzymes- of oxygen and free radicals
MT1H	Metallothionein-1H	4,100 + 85	binds various heavy metals
MT2H	Metallothionein-2H	200 + 14	binds various heavy metals
PCNA	Proliferating Cell Nuclear Antigen	780 + 20	DNA synthesis and repair, tissue repair
SOD1	Superoxide dismutase 1	160 + 6	anti-oxidant activity- of oxygen and free radicals
SOD2	Superoxide dismutase 2	160 + 7	anti-oxidant activity- of oxygen and free radicals
Growth factor			
NGF	Nerve growth factor	800 + 29	skin tissue repair
Aging			
S100 A8	S100 Calcium-binding Protein A8	-340 + 55	increases with age, pro-inflammatory/UV exposure
S100 A9	S100 Calcium-binding Protein A9	-290 + 75	increases with age, pro-inflammatory/UV exposure
Inflammatory			
IL1A	Interleukin 1 Alpha	-2,200 + 45	pro-inflammatory
IL1RII	Interleukin 1 Receptor 2	-590 + 15	pro-inflammatory
IL6	Interleukin 6	-3,200 + 105	pro-inflammatory
IL8	Interleukin 8	-790 + 17	pro-inflammatory
TNFRSF1A	Tumor Necrosis Factor Receptor 1A	-160 + 8	pro-inflammatory

Table 4: Human Skin-related Genes Modulated by Resveratrol (@1.0%) in EpiDermal Full Thickness Cultures.

<Significantly greater stimulation compared to R-equol; << Significantly greater stimulation compared to Racemic equol or R-equol; >Significantly greater stimulation compared to S-equol; >> Significantly greater stimulation compared to Racemic equol or S-equol; ■Significantly greater stimulation compared to R-equol; ♦Significantly greater inhibition compared to S-equol; ♦♦Significantly greater inhibition compared to R-equol or S-equol; ♣Significantly greater inhibition compared to Racemic equol or S-equol; ♣Significantly greater inhibition compared to Racemic equol or R-equol. 1.2% equol (treatments: R-equol, Racemic equol or S-equol) in DMSO vehicle vs. DMSO control levels; n=6. Values represent percentage (%) increase or decrease (-) vs. control values (mean + SEM) of gene array analysis (via quantification of mRNA levels by Taqman fluorescence detection), p<0.005 for all data. NA=not assayed. Data from references [14,25].

To summarize the results of this study [54], resveratrol at 1% in gene array/qPCR experiments using a human skin model (EFT) cultures significantly increased gene expression of: 1) the anti-aging factor, SIRT1, 2) the extracellular matrix proteins (collagens- COL1A1, COL3A1, COL1A1, ELN, LOX and TIMP1, etc.) and 3) several antioxidant biomarkers such as CAT, MT1H, MT2H, PCNA, SOD1, and SOD2, while at the same time, resveratrol significantly inhibited the gene expression of 4) proteinases of collagen/elastin, i.e., MMP1 and MMP9, 5) the aging and inflammatory genes (S100 A8 and A9; IL1A, IL1RII, IL6, IL8 and TNFRSF1A). These data sets are displayed in Table 4 [54].

Human Epidermal Full Thickness Cultures were incubated in the absence (DMSO vehicle) or presence of resveratrol (1%) plus DMSO for 24 hours, after which skin-related genes were quantified by gene array analysis (via quantification of mRNA levels by Taqman fluorescence detection), p<0.005. N=6 samples were assayed per biomarker in this study [54].

This recent study showed that resveratrol can ameliorate the aging of human skin by influencing the expression of human skin genes in a positive manner. The anti-aging protection provided by resveratrol involved enhancing specific dermal biomarkers through a variety of mechanisms that require further study to elucidate the multi-faceted

properties and actions of resveratrol in its applications to human dermal health.

Conclusions

The reviewed studies demonstrate how human skin genes are modulated by 1) enhanced wound healing with an extract of a well-known medicinal plant in Asia, *Angelica sinensis*, 2) UV sunlight exposure that represents the main cause of photo- or extrinsic skin aging and subsequent protection by a soy extract, 3) equol and their isomers that stimulate collagen and elastin while at the same time inhibited aging and inflammatory biomarkers and 4) resveratrol, the most high profile phytochemical known by the general public that displayed similar properties to equol with the additional benefit of stimulating the anti-aging sirtuin or SIRT1 biomarker.

In attempting to provide an analysis of comparison as to which phytochemical(s), in the case of the extracts, or individual compounds tested represents the best agent for human skin anti-aging, it is difficult to make a solid determination based upon the different experimental designs for the studies reviewed therein. But, in general, based upon the number and magnitude of biomarkers reported related to improved dermal health equol appears to represent the best agent for

efficacy among these observations. However, resveratrol, followed closely by the soy-extract and *Angelica sinensis* also demonstrated very good results for specific applications. Thus, all of these studies demonstrated the valuable aspects of gene array methodologies and elucidated the molecular regulation of human skin related-genes by the natural damaging effects of UV exposure and conversely, the protective influences of phytochemicals/botanicals that fall under, the polyphenolic class, for the most part, that provide applications to improve human skin health.

Acknowledgments

This study was supported, in part, by LS/TTO funding, 19-2215 at Brigham Young University.

References

- Blumenburg M (2006) DNA microarrays in dermatology and skin biology. *Omic* 10: 243-260.
- Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, et al. (2003) *Fitzpatrick's Dermatology in General Medicine* (6th edn), McGraw Hill, New York, USA.
- Burns DA, Breathnach SM (2004) *Rook's Textbook of Dermatology* (7th edn) Blackwell Publishing Co, Massachusetts, USA
- Baumann L (2002) *Cosmetic Dermatology: Principles and Practice* (1st edn) McGraw Hill, Hong Kong
- Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, et al. (2002) Plants and human health in the twenty-first century. *Trends Biotech* 20: 522-531.
- Goldman P (2001) Herbal medicines today and the roots of modern pharmacology. *Annual Inter Med* 135: 594-600.
- Halberstein RA (2005) Medicinal Plants: Historical and cross-cultural usage patterns. *Annual Epidemiol* 15: 686-699.
- Hunt KJ, Hung SK, Ernst E (2010) Botanical Extracts as Anti-aging Preparations for the Skin. *Drugs Aging* 27: 973-985.
- Perls TT (2006) Hope Drives Anti-aging Hype. *Cleveland Clin J Med* 73: 1039-1044.
- Thornefeldt C (2005) Cosmeceuticals Containing Herbs: Fact, Fiction and Future. *Dermatol Surg* 37: 873-880.
- Baumann LS (2007) Less-known Cosmeceuticals. *Dermatol Ther* 20: 339-342.
- Bogdan AI, Baumann L (2008) Antioxidants used in skincare formulations. *Skin Ther Lett* 13: 1-12.
- Khol E, Steinbauer J, Landthaler M, Szeimies RM (2011) Skin Aging *J European Acad Dermatol Venereol* 25: 873-884.
- Gopal R, Knaggs H, Lephart ED (2012) Biochemical investigation and gene analysis of equol: A plant and soy-derived isoflavonoid with anti-aging and anti-oxidant properties with potential human skin applications. *Biofactors* 38: 44-52.
- Tang QZ, Li YS, Huang CX, Xu JL (1995) Effect of Dang gui on the level of endothelin in patients with angina pectoris (Chinese). *J Chinese Western Traditional Combinatory Med* 15: 539-540.
- Liu KY, Zhang YQ, Zhang Y, Ma YM, Feng ZX (1997) Clinical study of Dang gui bu xie mixture on angina pectoris (Chinese). *J Anhui TCM College* 16: 17-18.
- Xia G (2001) Discussion of menstrual cycle and the cycle-regulating treatment. *J Chinese Med* 67: 30-34.
- Zhao H, Deneau J, Che GOL, Li S, Frederic V, et al. (2012) *Angelica sinensis* isolate SBD.4: composition, gene expression profiling, mechanism of action and effect of wounds, in rats and humans. *Eur J Dermatol* 22: 58-67.
- Zhoa H, Aria F, Martezaei R, Wang YR, Sheng XS, et al. (2006) SBD.4 Stimulates wound healing in genetically diabetic mice and in human skin/SCID mouse chimera. *Wound Repair Regen* 14: 593-601.
- Sudel KM, Venzke K, Mielke H, Breitenbach U, Mundt C, et al. (2005) Novel aspects of intrinsic and extrinsic aging of human skin: beneficial effects of soy extract. *Photochem Photobiol* 81: 581-587.
- Sudel KMK, Venzke E, Knussmann-Hartig E, Moll I, Stab H, et al. (2003) Tight control of matrix metalloproteinase-1 activity in human skin. *Photochem Photobiol* 78: 355-360.
- Setchell KDR, Clerici C (2010) Equol: History, Chemistry, and Formation. *J Nutr* 140: 1355S-1362.
- Lund TD, Blake C, Bu L, Hamaker AN, Lephart ED (2011) Equol an isoflavonoid: potential for improved prostate health, in vitro and in vivo evidence. *Reprod Biol Endocrinol* 9: 4.
- Blake C, Fabick KM, Setchell KDR, Lund TD, Lephart ED (2011) Neuromodulation by Soy Diet or Equol: Anti-depressive & Anti-obesity-like Influences, Age- & Hormone-dependent Effects. *BMC Neuroscience* 12: 28.
- Lephart ED (2013) Protective effects of equol and their polyphenolic isomers against dermal aging: Microarray/protein evidence with clinical implications and unique delivery into human skin. *Pharmaceutical Biol* 51: 1391-1400.
- Thornton MJ, Taylor AH, Mulligan K, Al-Azzawi F, Lyon CC, et al. (2003) Oestrogen receptor beta is the predominant oestrogen receptor in human scalp skin. *Exper Dermatol* 12: 181-190.
- Pelletier G, Ren L (2004) Localization of sex steroid receptors in human skin. *Histology Histopathology* 19: 629-636.
- Hirvonen J, Rajalin AM, Wohlfart G, Adlercreutz H, Wähälä K, Aarnisalo P (2011) Transcriptional activation of estrogen-related receptor gamma (ERR gamma) is stimulated by the phytoestrogen equol. *J Steroid Biochem Mole Biol* 123: 46-57.
- Krahn-Bertel E, Bolzinger MA, Andre V, Orly I, Kanitakis J, et al. (2008) Expression of estrogen-related receptor gamma (ERR gamma) in human skin. *Eur J Dermatol* 18: 427-432.
- Hounsome N, Hounsome B, Deri Tomos A, Edwards-Jones G (2009) Changes in anti-oxidant compounds in white cabbage during winter storage. *Postharvest Biol Tech* 52: 173-179.
- Hounsome N, Grail B, Deri Tomos A, Hounsome B, Edwards-Jones G (2010) High-throughput anti-oxidant profiling in vegetables by Fourier-transform ion Cyclotron resonance mass spectrometry. *Functional Plant Sci Biotech* 4: 1-10.
- Abiru Y, Kumenu M, Ueno T, Uchiyama S, Masaki K (2012) Discovery of an S-equol rich food stinky tofu, a traditional fermented soy product in Taiwan. *Internat J Food Sci Nutr* 63: 964-970.
- Jou HJ, Tsai PJ, Tu JH, Wu WH (2013) Stinky tofu as a rich source of bioavailable S-equol in Asian diets. *J Functional Foods* 5: 651-659.
- Setchell KDR, Clerici C, Lephart ED, Cole SJ, Heenan C, et al. (2005) S-Equol, a potent ligand for estrogen receptor β , is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am J Clin Nutr* 81: 1072-1079.
- Lephart ED (2013) Isoflavones and Prenatal Exposure to Equol. In: Preedy VR (ed) *Isoflavones: Chemistry, Analysis, Function and Effects*, The Royal Society of Chemistry, Thomas Graham House, Cambridge, England, United Kingdom.
- Setchell KDR, Zhao X, Jah P, Heubi JE, Brown NM (2009) The pharmacokinetic behavior of the soy isoflavone metabolite S(-) equol and its diastereoisomer R-(+)-equol in healthy adults determined by using stable-isotope-labeled tracers. *Am J Clin Nutr* 90: 1029-1037.
- Frankenfeld CL (2011) Dairy consumption is a significant correlate of urinary equol concentration in representative sample of US adults. *Am J Clin Nutr* 93: 1109-1116.
- Munro IC, Harwood M, Hlywka JJ, Stephen AM, Doull JD, et al. (2003) Soy Isoflavones: A Safety Review. *Nutr Rev*, 2003, 61, 1-33.
- Zhang T, Xinyu L, Shi L, Wang L, Chen J, et al. (2013) Estrogen receptor and PI3K/Akt signaling pathway involvement in S(-) equol-induced activation of Nrf2/ARE in endothelial cells. *Plos One* 8: e79075.
- Beyer TA, Keller U, Braun S, Schafer M, Werner S (2007) Roles and mechanisms of action of the Nrf2 transcription factor in skin morphogenesis, wound repair and skin cancer. *Cell Death Differentiation* 14: 1250-1254.
- Khurana S, Venkataraman K, Hollingsworth A, Piche M, Tai TC (2013) Polyphenols: Benefits to the cardiovascular system in health and in aging. *Nutrients* 5: 3779-3827.
- Meydani M, Hasan ST (2010) Dietary polyphenols and obesity. *Nutrients* 2: 737-751.
- Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, et al. (2014) Novel insights of dietary polyphenols and obesity. *J Nutr Biochem* 25: 1-18.
- Jang M., Cai L, Udeani GO, Slowing K, Thomas CF, et al. (1997) Cancer

- chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275: 218-220.
45. Kasiotis KM, Pratsinis H, Kletsas D, Haroutounian SA (2013) Resveratrol and related stilbenes: Their anti-aging and anti-angiogenic properties. *Food Chem Tox* 61: 112-120.
46. Kopec A, Piatkowska E, Leszczynska T, Koronowicz A (2013) Effect of long term administration of resveratrol on lipid concentration in selected organs and liver's histology in rats fed high fructose diet. *J Functional Foods* 5: 299-305.
47. Lam YY, Peterson CM, Ravussin E (2013) Resveratrol vs. calorie restriction: Data from rodents to humans. *Exper Gerontol* 48: 1018-1024.
48. Pezzuto JM (2011) The phenomenon of resveratrol: redefining the virtues of promiscuity. *Ann NY Acad Sci* 1215: 123-130.
49. Smoliga JM, Baur JA, Hausenblas HA (2011) Resveratrol and health- A comprehensive review of human clinical trials. *Molec Nutr Food Res* 55: 1129-1141.
50. Tome-Carnerio J, Larrosa M, Gonzalez-Sarrias A, Tomas-Barberan A, Garcia-Conesa MT, et al. (2013) Resveratrol and clinical trials: The crossroad from in vitro studies to human evidence. *Curr Pharm Design* 19: 6064-6093.
51. Turan B, Tuncay E, Vassort G (2012) Resveratrol and diabetic cardiac function: focus on recent in vitro and in vivo studies. *J Bioeng Biomemb* 44: 281-296.
52. Baumann LS (2009) *Alternative Medicine. Cosmetic Dermatology: Principles and Practice* (2nd edn), McGraw-Hill: Columbus, Ohio, USA.
53. Evans JA, Johnson EJ (2010) The role of phytonutrients in skin health. *Nutrients* 2: 903-928.
54. Lephart ED, Sommerfeldt JM, Andrus MB (2014) Resveratrol: influences on gene expression in human skin. *J Functional Foods* 10: 377-384.