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The mechanism of action and clinical benefits of soy for the treatment of hyperpigmentation

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Abstract

Background Hyperpigmentation disorders are common and diverse conditions that may require treatment for medical and/or cosmetic reasons. Hyperpigmented lesions can reduce patients' quality of life, self-perception, and social and vocational functioning. The most commonly used treatments for hyperpigmentation include topical agents, such as hydroquinone, retinoids and azelaic acid.

Objectives Current topical treatments have significant limitations; they often do not produce adequate results and may be limited by adverse effects, such as dermatitis. Soy and soy-based products have demonstrated a wide range of potential benefits for health and nutrition, including a range of dermatological effects.

Methods Research from the last decade has identified multiple mechanisms by which soy-derived products may affect skin pigmentation, as well as photodamage and photoaging, overall skin health, and even the risk for and progression of skin cancer.

Results Preclinical evidence has demonstrated that soy-derived serine protease inhibitors affect skin pigmentation by inhibiting protease-activated receptor-2-mediated phagocytosis of melanosomes by keratinocytes.

Conclusion Soy-based products containing these serine protease inhibitors may represent a new therapeutic option for dermatological treatment. Indeed, recent evidence from randomized clinical studies supports the safe and effective use of soy products for the treatment of hyperpigmentation.

Introduction

Hyperpigmentation disorders are common, encompassing multiple types and etiologies. These disorders are often benign, but can have a significant impact on psychosocial functioning and self-perception. Topical agents are the most common form of treatment for hyperpigmentation. These agents include hydroquinone, retinoids, azelaic acid, kojic acid and others, as well as combinations of agents.

Over the last 10 years, a wide range of dermatological and other benefits associated with soy-based products has been reported in the literature. Proposed health benefits of soy range from effects on carcinogenesis, reduced risk for heart disease, modulation of peri-menopausal symptoms, and a multitude of effects in skin.[1–3] Indeed, mounting evidence suggests a role for soy-based products in photoprotection, the amelioration of age-associated skin changes, such as wrinkles and reduced elasticity, and the treatment of hyperpigmentation.[1–10] This article reviews the evidence supporting the use of soy-based products for the treatment of hyperpigmentation and the mechanisms by which these agents may act.

Hyperpigmentation

Hyperpigmentation of the skin is a common disorder that results from an increase in cutaneous melanin deposition, either through increased melanin synthesis or increased numbers of melanocytes.[11] Hyperpigmentation may result from increased production of melanosomes in response to stimuli such as irritation or hormones; conversely, hyperpigmentation may result from hyperplasia of melanocytes in response to sun exposure or as a result of some idiopathic disorders.[12]

The location of increased melanin deposition affects the type of color change and choice of treatment. Increased melanin in the epidermal layer often appears brown, compared with blue-gray when located in the dermal layer or brown-gray when both layers are involved.[11,13] Because most therapies for hyperpigmentation are topical, involvement of the dermal layer can be much more difficult to treat compared with epidermal involvement.[14]

Treating hyperpigmentation: available therapies

The treatment of hyperpigmentation may be appropriate for cosmetic and/or medical reasons. The goals of treating hyperpigmentation include inhibiting the formation and promoting the degradation of melanosomes, as well as inhibiting the proliferation of melanocytes.[11] Therapies commonly used for hyperpigmentation of the epidermal layer include topical agents (Table 1), as well as dermabrasion and laser therapy. Topical agents are the most widely used therapy.[11]

The gold standard for topical therapy is hydroquinone, a phenolic compound that inhibits the tyrosinase enzyme, thereby reducing the production of melanin.[15] It is often used in combination with a retinoid and a topical corticosteroid. The combination of hydroquinone, tretinoin and dexamethasone, for example, has been widely used for hyperpigmentation since its introduction in 1975.[11] The retinoids tretinoin and tazarotene have also demonstrated pigment-lightening activity. Tretinoin acts through inhibition of tyrosinase transcription and disruption of melanin synthesis.[11] Retinoids cause cutaneous changes that allow for better penetration of active depigmenting agents, and are often used in combination therapy, as described above.[15,16] Another topical agent used for pigment lightening is azelaic acid, a reversible inhibitor of tyrosinase derived from *Malassezia*.[17] Several other compounds have been used for pigment lightening, including glycolic acid, kojic acid, arbutin, aleosin, licorice extract, N-acetyl glucosamine and ascorbic acid.[15,17] Only limited data are available describing the efficacy of these agents for hyperpigmentation.

There is a clear and outstanding need for more effective, safer and more tolerable topical agents for the treatment of hyperpigmentation.

Table 1 Topical agents used for treatment of hyperpigmentation[11,15–17]

Class	Agent(s)
Phenols	Hydroquinone
	Mequinol
	Rucinol
Retinoids	Tretinoin
	Tazarotene
Other agents	Ascorbic acid
	Azelaic acid
	Kojic acid
	Glycolic acid
	N-acetyl glucosamine
	Arbutin
	Aleosin
	Licorice extract
	Mulberry extract
	Pomegranate extract
	Soy proteins
	Vitamin E
Combinations	Azelaic acid, hydroquinone
	Glucosamine, niacinamide
	Glycolic acid, hydroquinone
	Hydroquinone, tretinoin, topical corticosteroid
	Kojic acid, hydroquinone
	Mequinol, tretinoin, vitamin C
	Retinol, hydroquinone
	Salicylic acid, hydroquinone

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Soy-based products and skin health

For centuries, soy has been associated with health and nutrition, and growing evidence supports a role for soy-based products in skin care. Consumption of soy food products may contribute to reduced risk for cardiovascular disease,[18,19] diabetes,[20,21] and breast[22] and prostate cancer.[23,24] Several compounds found in soy have been studied for the promotion of skin health.[1–5,7–9,25–27] Soy isoflavones are the most-studied biologically active compounds found in soybeans; these compounds include genistein, daidzin and glycitein.[28] Serine protease inhibitors, including soybean trypsin inhibitor (STI) and Bowman-Birk protease inhibitor (BBI), are also found in soybeans. Unlike isoflavones, however, the serine protease inhibitors are generally inactivated during the processing of soybeans for consumption.[10]

Both soy isoflavones and serine protease inhibitors have been associated with potential benefits for skin (Table 2). Isoflavones, particularly genistein, have multiple effects on skin and skin cells. In preclinical studies, preparations containing isoflavones have demonstrated antioxidant activity, the ability to inhibit tyrosine kinase function, multiple effects on skin cell growth and proliferation, response to ultraviolet (UV) exposure, and maintenance of extracellular matrix.[26,27] For example, genistein has been shown to reverse the inhibitory effects of oxidative stress on collagen synthesis *in vitro*, although higher concentrations of the isoflavone may actually inhibit collagen biosynthesis.[27] A molecular biological study reported that

Table 2 Soy compounds associated with potential benefits for skin[1–5,7,8,25,27,28,32,51]

Family	Examples	Activity	Potential benefits for skin
Serine protease inhibitors	Soybean trypsin inhibitor (STI) Bowman-Birk protease inhibitor (BBI)	Protease-activated receptor-2 (PAR-2) inhibition	Depigmentation Treatment of photodamage
Isoflavones	Genistein Daidzin Glycitein Aglycone	Antioxidant activity Tyrosine kinase inhibition Block UV-induced mitogen-activated protein (MAP) kinase signal transduction Preserve cell proliferation and repair mechanics Stimulate hyaluronic acid production Reduced collagen degradation Attenuation of retinoid-induced epidermal hyperplasia	Photoprotection Improved elasticity Improved viscoelasticity Reduced wrinkles
Non-denatured soy extracts		Inhibition of UVB-induced thymine–thymine dimer formation Anti-inflammatory activity	Prevention or reduction of UVB-induced skin damage Reduced initiation and progression of skin cancer

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non-denatured soy extracts induced activity of the elastin promoter and inhibited activity of elastase in a cultured cardiac myoblast cell line.[29] Elastin is a key contributor to the elasticity of skin, and is degraded by elastases, which are induced by aging, UV exposure and inflammation. Other studies have demonstrated that treatment of skin with soy isoflavones can significantly reduce UV-induced epidermal thickening as well as measures of skin roughness.[8] Intriguing results suggest that topical application of non-denatured soy milk can inhibit the formation and growth of skin tumors.[5] Application of isolated STI and BBI also inhibited tumor formation and growth, but to a lesser degree compared with the soy milk preparation.[5]

In human studies, use of soy isoflavones improved elasticity and viscoelasticity, reduced wrinkling of the skin, and led to increased numbers of dermal papillae.[1,7,9] One study in humans demonstrated that genistein can interrupt the UV signaling cascade that is involved in photoaging through inhibition of UV-stimulated activation of epidermal growth factor receptor, mitogen-activated protein kinase phosphorylation of JNK, collagenase mRNA induction and other mechanisms.[9] Even oral intake of the isoflavone aglycone has been shown to significantly improve fine wrinkles and skin elasticity in human subjects.[7] In these human studies, soy-based products demonstrated excellent safety and tolerability.

Mechanisms of skin pigmentation: therapeutic targets for soy-based products

Skin pigmentation derives from the multistep conversion of tyrosine to melanin in melanosomes. These organelles are produced continually by melanocytes and extruded for incorporation by neighboring keratinocytes.[12,30] Once incorporated by keratinocytes, the melanosomes are trafficked to the apical pole of the cell where they serve to protect the nucleus by absorbing ultraviolet (UV) light.[30] The functional unit of the epidermis responsible for this activity is the epidermal-melanin unit, which consists of approximately one melanocyte and 36 keratinocytes.[31] Early microscopic studies suggested multiple potential mechanisms by which melanosomes could be transferred from melanocytes to keratinocytes. Subsequent studies demonstrated that keratinocytes ingest melanosomes through phagocytosis, a mechanism more commonly associated with immune cells, such as macrophages, neutrophils and monoctyes.[31] The process of phagocytosis is a key therapeutic target for soy-based products.

Melanosome regulation: role of protease-activated receptor-2 (PAR-2)

A key mediator of the transfer of melanosomes from melanocytes to keratinocytes is the PAR-2. PAR-2 is expressed on the cell surface of keratinocytes, but is not expressed on melanocytes.[32] Initial evidence of PAR-2 activity in keratinocytes was derived from studies of thrombin receptor activation. Among its many actions, the serine protease thrombin is involved in epidermal response to injury, including the stimulation of fibroblast proliferation and collagen synthesis.[33] Early evidence suggested an effect of thrombin on epidermal keratinocytes as well, and *in vitro* studies were undertaken to examine the effects of thrombin, trypsin and receptor agonist

peptides on inositol phospholipid hydrolysis and calcium homeostasis.[34] These studies revealed a potent effect of trypsin on keratinocytes, and led to the hypothesis that keratinocytes express the trypsin-sensitive receptor PAR-2.

PAR-2 is a seven-transmembrane G-protein-coupled receptor related to the family of thrombin receptors.[31] Serine proteases cleave a portion of the extracellular domain of PAR-2, revealing a new N-terminus that acts as a tethered ligand for PAR-2 activation (Fig. 1). Synthetic peptides corresponding to this exposed N-terminal domain have been created for mouse and human PAR-2, and can be used to activate the receptors without the need for proteolytic cleavage.[31,34] Multiple cell types express PARs, and the receptors are known to be involved in a wide range of processes, including cell growth, and development and regulation of inflammation.[31] PAR-2 activation in keratinocytes produces multiple changes characteristic of phagocytosis, including cytoskeletal reorganization and specific morphological changes on the cell surface.[35] Cultured keratinocyte cell lines also demonstrate increased phagocytosis of fluorescently labeled microspheres and *Escherichia coli* bioparticles in response to activation by trypsin or the PAR-2-activating peptide SLIGRL.[35] The Rho family of GTP-binding proteins plays a central role in the cytoskeletal reorganization that occurs during phagocytosis. Using specific inhibitors of Rho kinase, investigators have demonstrated that PAR-2-mediated phagocytosis is Rho-dependent in cultured human keratinocytes, and that activation of PAR-2 leads to activation of Rho and cyclic adenosine monophosphate (cAMP).[36] In this study, inhibition of Rho did not alter PAR-2 mRNA, protein or activity. Other authors reported that stimulation of PAR-2 led to increased secretion and activation of soluble serine proteases, potentially representing a positive feedback mechanism contributing to enhanced activation of PAR-2.[35]

The involvement of PAR-2 in melanosome transfer and pigmentation was demonstrated through studies of melanocyte and keratinocyte co-cultures called epidermal equivalents. Exposure of these cultures to UVB radiation increased the number of pigment granules in melanocytes; the opposite effect was produced by addition of the depigmenting agent benzaldehyde.[37] Treatment with trypsin produced a similar effect to UVB, whereas treatment with STI decreased pigmentation similarly to benzaldehyde.[37] The addition of the peptide SLIGRL, which specifically activates PAR-2, also produced pigmentation in the epidermal equivalents, indicating that increased melanosome transfer is related specifically to this receptor.[37] Furthermore, the serine protease inhibitor RWJ-50353 inhibited the pigmentation induced by UVB radiation, suggesting that the effects of UVB on pigmentation may be regulated in part by PAR-2 activation.[37] In a different study, cultured keratinocytes were pretreated with either SLIGRL or RWJ-50353, and then incubated with isolated melanosomes. Increased uptake of melanosomes was observed following SLIGRL treatment and decreased uptake following RWJ-50353 treatment.[38]

Electron microscopic analysis of co-cultures treated with RWJ-50353 revealed that the melanosomes were less mature and increased in number within melanocytes compared with untreated control cultures. Furthermore,

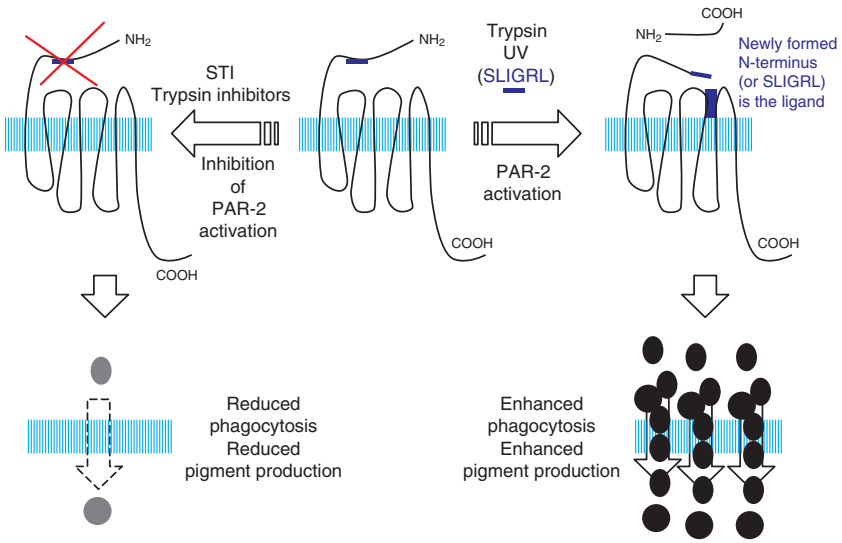

Figure 1 PAR-2 activation and inhibition, and influence on melanosome transfer and pigmentation. PAR-2, protease-activated receptor-2; STI, soybean trypsin inhibitor. Reprinted with permission from Seiberg. *Pigment Cell Res.* 2001; 14: 236–242

there were more melanosomes in the dendrites of treated keratinocytes compared with control cultures. Together, these findings suggest that PAR-2 inhibition leads to abnormal melanosome function and impaired melanosome transfer into keratinocytes.[38] Cell-to-cell contact appears to be important for these PAR-2-related effects. Increased pigmentation resulting from application of trypsin and SLIGRL and depigmentation in association with RWJ-50353 occurred only in two- and three-dimensional co-cultures, and not in cultures in which keratinocytes and melanocytes were separated.

In vivo studies describe a similar role for PAR-2 on pigmentation. Application of SLIGRL to human skin results in visible darkening and increased deposition of melanin in keratinocytes.[37] Conversely, application of RW-50353 results in a visible whitening effect when applied to swine skin for 4 weeks; this effect occurs without signs of inflammation and is reversible following discontinuation of treatment.[38] The involvement of PAR-2 in melanosome transfer suggests that this receptor may play a role in normal skin darkening following exposure to sunlight. Indeed, examination of human skin biopsies following exposure to UV radiation revealed increased PAR-2 expression compared with non-irradiated skin.[39] Similar results were found in cultured human keratinocytes following exposure to UV radiation. Analysis of the supernatants from these cultures revealed a dose-related increase in protease activity and specific PAR-2 cleavage activity.[39]

Interestingly, the effects of UV radiation on PAR-2 expression in human skin samples were related to skin type. The expression of PAR-2 was significantly increased only in skin samples from individuals with type II or III skin – skin types that tan more easily – and was not significantly increased in type I skin samples.[39] Other investigators have demonstrated that the expression of both PAR-2 and trypsin is greater in highly pigmented compared with lightly pigmented skin types.[40] In light and medium color skins (i.e. type I or II), PAR-2 was expressed only in the keratinocytes of the lower third of the epidermis; in dark skin, PAR-2 was expressed throughout the epidermis.[40] Levels of trypsin expression, proteolytic activity against PAR-2, and PAR-2-induced phagocytic activity all correlated with skin color.

PAR-2 has also been associated with inflammatory response during tissue injury,[41] and it has been suggested that the receptor could play a role in conditions such as post-inflammatory hyperpigmentation. Increased expression of PAR-2 and trypsin in the epidermis of dark-skinned individuals could relate to the higher rate of post-inflammatory hyperpigmentation described in this population.[40] With regard to the inflammatory processes associated with PAR-2 activation, an interesting recent report noted that the peptide LIGR (a shorter version of SLIGRL) stimulated skin pigmentation but not inflammatory processes.[42] This shorter peptide activated only a subset of PAR-2 signaling pathways, including induction of phagocytosis, but did not induce secretion of proinflammatory cytokines, prostaglandin E_2 or other mediators of inflammation.[42] PAR-2 may also regulate pigmentation through a paracrine effect on melanocytes. One study reported that stimulation of PAR-2 in keratinocytes led to the release of prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$). The authors further noted that PGE_2 and $PGF_{2\alpha}$ stimulated melanocyte dendricity in a cAMP-independent manner.[43]

PAR-2 and regulation of apoptosis

Several lines of evidence suggest that PAR-2 may be involved in the regulation of cell growth and development. For example, soy milk, STI and BBI have each been shown to reduce hair pigmentation and growth rate, and the dimensions of the hair follicle and shaft.[44] These effects appeared to be independent of papillae cell death, suggesting that serine protease inhibitors do not affect hair growth through increased apoptosis. Conversely, previous studies reported that the application of trypsin induced apoptosis of the follicular papilla, and resulted in delayed hair growth and pigmentation.[45] Indeed, a number of studies have implicated serine proteases in the apoptosis of keratinocytes and other skin cells.[45–47] Apoptosis, or programmed cell death, is centrally involved in the differentiation of basal keratinocytes into cornified cells, and in the normal cycle of hair follicle growth and regression. Trypsin has been shown to modulate apoptosis in various experimental models of skin. For example, the apoptosis of the epidermal keratinocyte cell line Pam212, a model of epidermal differentiation, can be induced by trypsin and blocked by STI.[47] Similarly, trypsin has been shown to induce apoptosis in the follicular papilla *in vivo*, resulting in delayed hair growth and pigmentation.[45] The dynamics of this effect suggested a possible receptor-mediated mechanism, for which PAR-2 is a likely candidate. Conversely, trypsin application in a model of acne characterized by extensive programmed cell death resulted in reduced apoptosis and improved biomechanical properties of the skin.[46] Together, these findings suggest a role for PAR-2 in the regulation of cell growth and development, and in the balance between survival and apoptosis.

Soy and the treatment of hyperpigmentation: clinical evidence

The utility of soy for the treatment of hyperpigmentation derives from the activity of the serine protease inhibitors

STI and BBI. These inhibitors have been shown to inhibit the activity of PAR-2, reduce keratinocyte phagocytosis, affect cytoskeletal organization and reduce pigmentation.[32] In one study, isolated STI and BBI were shown to reduce pigment deposition in keratinocyte-melanocyte co-cultures *in vitro*.[32] The same study also demonstrated that STI, as well as freshly prepared soy milk, reduced experimentally induced keratinocyte phagocytosis. Microscopic analysis of cell cultures revealed reduced number and length of cell podia following STI treatment, providing further evidence that soy serine protease inhibitors affect pigmentation by reducing the transfer of melanosomes into keratinocytes. Interestingly, both STI and soybean extracts reduced pigmentation and prevented UV-induced pigmentation *in vivo*.

Initial clinical evidence supports the use of soy-based topical products containing STI and/or BBI for the treatment of hyperpigmentation.[6,10,48,49] One study evaluated the use of moisturizers containing a total soy complex in 28 women with moderate levels of skin roughness, hyperpigmentation, blotchiness and dullness.[50] Twice-daily application of soy-containing product was associated with significant improvements in dermatologists' assessments of pigmentation, radiance, texture, blotchiness and sallowness. Improvements from baseline were statistically significant within 4 weeks of treatment ($P < 0.05$), and progressed throughout the 12-week study. Subject self-assessments and visible and enhanced photography also reflected improvements in skin tone, texture and radiance compared with baseline.

A more recent, randomized, double-blind, controlled study compared the efficacy of a novel soy moisturizer containing non-denatured STI and BBI to vehicle in 68 women with moderate facial photodamage.[10] Sixty-three women completed the 12-week study, during which time they applied active or vehicle product twice daily to their entire face. Both groups demonstrated significant improvements compared with baseline in all efficacy parameters except blotchiness, which was improved only in the active treatment group. The active treatment, however, produced significantly greater improvements in mottled pigmentation, blotchiness, dullness, fine lines, overall texture, overall skin tone and overall appearance compared with vehicle ($P \leq 0.05$). For most parameters, the difference between groups was significant by study week 2. Specifically with regard to hyperpigmentation, 28 of 31 subjects in the active treatment group showed improvement of at least one grade in mottled hyperpigmentation, compared with 17 of 32 subjects in the vehicle group. The active treatment group also showed significant improvement in subject self-assessment of skin imperfections as early as week 1 ($P \leq 0.05$ versus baseline). Both formulations were well tolerated.

Similar results have been reported by other investigators.[6,48,49] One group reported that evaluation of a soy-based product containing STI demonstrated significant improvements in hyperpigmented lesions and reductions in postinflammatory hyperpigmentation, and desquamation and erythema following UV exposure.[6]

Conclusions

Hyperpigmentation disorders represent a diverse spectrum of conditions, including postinflammatory hyperpigmentation, melasma, ephelides and lentigines. Soy-derived products have demonstrated numerous dermatological benefits *in vitro* and *in vivo*, ranging from depigmentation to prevention of photodamage and photoaging, to the potential to reduce the risk for and progression of skin cancer. Preclinical studies demonstrated that non-denatured soy products containing soy-derived serine protease inhibitors reduce pigmentation by inhibiting PAR-2-mediated phagocytosis of melanosomes by keratinocytes. PAR-2 appears to play a role in multiple aspects of skin biology, including pigmentation, inflammatory response, and cell growth and development. Together, the evidence indicates that PAR-2 is an excellent candidate target for therapeutic intervention. Initial clinical evidence supports the safe and effective use of soy products for hyperpigmentation. Future clinical study is required to better delineate the mechanisms, effects, and benefits of soy for dermatological applications.

References

1 Südel KM, Venzke K, Mielke H, *et al.* Novel aspects of intrinsic and extrinsic aging of human skin: beneficial effects of soy extract. *Photochem Photobiol* 2005; **81**: 581–587.
2 Moore JO, Wang Y, Stebbins WG, *et al.* Photoprotective effect of isoflavone genistein on ultraviolet B-induced pyrimidine dimer formation and PCNA expression in human reconstituted skin and its implications in dermatology and prevention of cutaneous carcinogenesis. *Carcinogenesis* 2006; **27**: 1627–1635.
3 Chen N, Scarpa R, Zhang L, *et al.* Nondenatured soy extracts reduce UVB-induced skin damage via multiple mechanisms. *Photochem Photobiol* 2008; **84**: 1551–1559.
4 Miyazaki K, Hanamizu T, Sone T, *et al.* Topical application of Bifidobacterium-fermented soy milk extract containing genistein and daidzein improves rheological and physiological properties of skin. *J Cosmet Sci* 2004; **55**: 473–479.
5 Huang MT, Xie JG, Lin CB, *et al.* Inhibitory effect of topical applications of nondenatured soymilk on the formation and growth of UVB-induced skin tumors. *Oncol Res* 2004; **14**: 387–397.

6 Liu J-C, Seiberg M, Miller J, *et al.* Soy: potential applications in skin care. Poster presented at: *59th Annual Meeting of the American Academy of Dermatology*, Washington, DC, March 2001.
7 Izumi T, Saito M, Obata A, *et al.* Oral intake of soy isoflavone aglycone improves the aged skin of adult women. *J Nutr Sci Vitaminol (Tokyo)* 2007; **53**: 57–62.
8 Kim SY, Kim SJ, Lee JY, *et al.* Protective effects of dietary soy isoflavones against UV-induced skin-aging in hairless mouse model. *J Am Coll Nutr* 2004; **23**: 157–162.
9 Kang S, Chung JH, Lee JH, *et al.* Topical N-acetyl cysteine and genistein prevent ultraviolet-light-induced signaling that leads to photoaging in human skin in vivo. *J Invest Dermatol* 2003; **120**: 835–841.
10 Wallo W, Nebus J, Leyden JJ. Efficacy of a soy moisturizer in photoaging: a double-blind, vehicle-controlled, 12-week study. *J Drugs Dermatol* 2007; **6**: 917–922.
11 Lynde CB, Kraft JN, Lynde CW. Topical treatments for melasma and postinflammatory hyperpigmentation. *Skin Therapy Lett* 2006; **11**: 1–6.
12 Stulberg DL, Clark N, Tovey D. Common hyperpigmentation disorders in adults: Part I. Diagnostic approach, café au lait macules, diffuse hyperpigmentation, sun exposure, and phototoxic reactions. *Am Fam Physician* 2003; **68**: 1955–1960.
13 Weismann K, Lorentzen HF. Dermoscopic color perspective. *Arch Dermatol* 2006; **142**: 1250.
14 Cayce KA, Feldman SR, McMichael AJ. Hyperpigmentation: a review of common treatment options. *J Drugs Dermatol* 2004; **3**: 668–673.
15 Draelos ZD. Skin lightening preparations and the hydroquinone controversy. *Dermatol Ther* 2007; **20**: 308–313.
16 Alexis AF, Roberts WE, Shah SK. Cosmeceuticals for hyperpigmentation. In: Sadick NS, Lupo MP, Berson DS, *et al.*, eds. *Cosmeceutical Science in Clinical Practice*. New York, NY: Informa Healthcare, 2010: 75–81.
17 Halder RM, Richards GM. Topical agents used in the management of hyperpigmentation. *Skin Therapy Lett* 2004; **9**: 1–3.
18 Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* 1995; **333**: 276–282.
19 Anthony MS, Clarkson TB, Williams JK. Effects of soy isoflavones on atherosclerosis: potential mechanisms. *Am J Clin Nutr* 1998; **68**: 1390S–1393S.
20 Ascencio C, Torres N, Isoard-Acosta F, *et al.* Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. *J Nutr* 2004; **134**: 522–529.
21 Nanri A, Mizoue T, Takahashi Y, *et al.* Soy product and isoflavone intakes are associated with a lower risk of type 2 diabetes in overweight Japanese women. *J Nutr* 2010; **140**: 580–586.
22 Shu XO, Zheng Y, Cai H, *et al.* Soy food intake and breast cancer survival. *JAMA* 2009; **302**: 2437–2443.
23 Hwang YW, Kim SY, Jee SH, *et al.* Soy food consumption and risk of prostate cancer: a meta-analysis of observational studies. *Nutr Cancer* 2009; **61**: 598–606.
24 Yan L, Spitznagel EL. Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. *Am J Clin Nutr* 2009; **89**: 1155–1163.
25 Sharma S, Sultana S. Modulatory effect of soy isoflavones on biochemical alterations mediated by TPA in mouse skin model. *Food Chem Toxicol* 2004; **42**: 1669–1675.
26 Miyazaki K, Hanamizu T, Iizuka R, *et al.* Bifidobacterium-fermented soy milk extract stimulates hyaluronic acid production in human skin cells and hairless mouse skin. *Skin Pharmacol Appl Skin Physiol* 2003; **16**: 108–116.
27 Sienkiewicz P, Surażyński A, Palka J, *et al.* Nutritional concentration of genistein protects human dermal fibroblasts from oxidative stress-induced collagen biosynthesis inhibition through IGF-I receptor-mediated signaling. *Acta Pol Pharm* 2008; **65**: 203–211.
28 Xiao CW. Health effects of soy protein and isoflavones in humans. *J Nutr* 2008; **138**: 1244S–1249S.
29 Zhao R, Bruning E, Rossetti D, *et al.* Extracts from *Glycine max* (soybean) induce elastin synthesis and inhibit elastase activity. *Exp Dermatol* 2009; **18**: 883–886.
30 Hershkovitz D, Sprecher E. Monogenic pigmentary skin disorders: genetics and pathophysiology. *Isr Med Assoc J* 2008; **10**: 713–717.
31 Seiberg M. Keratinocyte-melanocyte interactions during melanosome transfer. *Pigment Cell Res* 2001; **14**: 236–242.
32 Paine C, Sharlow E, Liebel F, *et al.* An alternative approach to depigmentation by soybean extracts via inhibition of the PAR-2 pathway. *J Invest Dermatol* 2001; **116**: 587–595.
33 Chambers RC, Dabbagh K, McAnulty RJ, *et al.* Thrombin stimulates fibroblast procollagen production via proteolytic activation of protease-activated receptor 1. *Biochem J* 1998; **333** (Pt 1): 121–127.
34 Santulli RJ, Derian CK, Darrow AL, *et al.* Evidence for the presence of a protease-activated receptor distinct from the thrombin receptor in human keratinocytes. *Proc Natl Acad Sci USA* 1995; **92**: 9151–9155.
35 Sharlow ER, Paine CS, Babiarz L, *et al.* The protease-activated receptor-2 upregulates keratinocyte phagocytosis. *J Cell Sci* 2000; **113** (Pt 17): 3093–3101.
36 Scott G, Leopardi S, Parker L, *et al.* The proteinase-activated receptor-2 mediates phagocytosis in a Rho-dependent manner in human keratinocytes. *J Invest Dermatol* 2003; **121**: 529–541.
37 Seiberg M, Paine C, Sharlow E, *et al.* The protease-activated receptor 2 regulates pigmentation via keratinocyte-melanocyte interactions. *Exp Cell Res* 2000; **254**: 25–32.
38 Seiberg M, Paine C, Sharlow E, *et al.* Inhibition of melanosome transfer results in skin lightening. *J Invest Dermatol* 2000; **115**: 162–167.

39 Scott G, Deng A, Rodriguez-Burford C, *et al.* Protease-activated receptor 2, a receptor involved in melanosome transfer, is upregulated in human skin by ultraviolet irradiation. *J Invest Dermatol* 2001; **117**: 1412–1420.
40 Babiarz-Magee L, Chen N, Seiberg M, *et al.* The expression and activation of protease-activated receptor-2 correlate with skin color. *Pigment Cell Res* 2004; **17**: 241–251.
41 Kanke T, Takizawa T, Kabeya M, *et al.* Physiology and pathophysiology of proteinase-activated receptors (PARs): PAR-2 as a potential therapeutic target. *J Pharmacol Sci* 2005; **97**: 38–42.
42 Lin CB, Chen N, Scarpa R, *et al.* LIGR, a protease-activated receptor-2-derived peptide, enhances skin pigmentation without inducing inflammatory processes. *Pigment Cell Melanoma Res* 2008; **21**: 172–183.
43 Scott G, Leopardi S, Printup S, *et al.* Proteinase-activated receptor-2 stimulates prostaglandin production in keratinocytes: analysis of prostaglandin receptors on human melanocytes and effects of PGE2 and PGF2alpha on melanocyte dendricity. *J Invest Dermatol* 2004; **122**: 1214–1224.
44 Seiberg M, Liu JC, Babiarz L, *et al.* Soymilk reduces hair growth and hair follicle dimensions. *Exp Dermatol* 2001; **10**: 405–413.
45 Seiberg M, Wisniewski S, Cauwenbergh G, *et al.* Trypsin-induced follicular papilla apoptosis results in delayed hair growth and pigmentation. *Dev Dyn* 1997; **208**: 553–564.
46 Seiberg M, Siock P, Wisniewski S, *et al.* The effects of trypsin on apoptosis, utriculi size, and skin elasticity in the Rhino mouse. *J Invest Dermatol* 1997; **109**: 370–376.
47 Marthinuss J, Andrade-Gordon P, Seiberg M. A secreted serine protease can induce apoptosis in Pam212 keratinocytes. *Cell Growth Differ* 1995; **6**: 807–816.
48 Hermanns JF, Petit L, Pierard-Franchimont C, *et al.* Assessment of topical hypopigmenting agents on solar lentigines of Asian women. *Dermatology* 2002; **204**: 281–286.
49 Hermanns JF, Petit L, Martalo O, *et al.* Unraveling the patterns of subclinical pheomelanin-enriched facial hyperpigmentation: effect of depigmenting agents. *Dermatology* 2000; **201**: 118–122.
50 Nebus J, Wallo W, Sher D, *et al.* Clinical improvement in skin tone, texture and radiance with facial moisturizers containing total soy complex. Poster presented at: *20th World Congress of Dermatology*, Paris, France, July 2002.
51 Varani J, Kelley EA, Perone P, *et al.* Retinoid-induced epidermal hyperplasia in human skin organ culture: inhibition with soy extract and soy isoflavones. *Exp Mol Pathol* 2004; **77**: 176–183.

