Green tea and the skin

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Plant extracts have been widely used as topical applications for wound-healing, anti-aging, and disease treatments. Examples of these include ginkgo biloba, echinacea, ginseng, grape seed, green tea, lemon, lavender, rosemary, thuja, sarsaparilla, soy, prickly pear, sagebrush, jojoba, aloe vera, allantoin, feverwort, bloodroot, apache plume, and papaya. These plants share a common character: they all produce flavonoid compounds with phenolic structures. These phytochemicals are highly reactive with other compounds, such as reactive oxygen species and biologic macromolecules, to neutralize free radicals or initiate biological effects. A short list of phenolic phytochemicals with promising properties to benefit human health includes a group of polyphenol compounds, called catechins, found in green tea. This article summarizes the findings of studies using green tea polyphenols as chemopreventive, natural healing, and anti-aging agents for human skin, and discusses possible mechanisms of action. (J Am Acad Dermatol 2005;52:1049-59.)

A 5-year skin-cancer prevention and education campaign sponsored by the US Department of Health and Human Services and the Centers for Disease Control and Prevention (CDC) ended in May 2003. During this period, skin cancer incidence in the United States had climbed to more than 1 million cases per annum, rendering skin cancer the most common type of neoplasm in the United States.[1] Unfortunately, unprotected ultraviolet (UV) exposure, the most preventable risk factor, continues to cause increasing numbers of skin cancers in the population. Because of the rapid increase in skin cancer incidence, the search for nontoxic and effective agents to protect the skin against solar irradiation (ie, UVA, UVB) has accelerated during the past 2 decades. Scientists searching for more active approaches to protect skin using plant-derived compounds identified the polyphenolic fraction of green tea as a prime candidate.[2-4] Tea, the second-most popular beverage next to water, was found to be beneficial to the skin when applied topically. There have been more than 150 reports of in vivo and in vitro studies on the effects of green tea on the skin (PubMed search; key words "green tea" and "skin"). The early focus of these studies was chemoprevention of chemical carcinogenesis or photocarcinogenesis in rodents. It was found that green tea extracts or an individual green tea polyphenol (GTPP), especially (−)-epigallocatechin (EGC)-3-gallate (EGCG), inhibited two-stage chemical carcinogenesis (eg, induced by 7,12-dimethylbenz(a)anthracene [DMBA] and 12-O-tetradecanoylphorbol 13-acetate [TPA]), and photocarcinogenesis (induced by UVB).[5] In addition to skin, studies in rodent models demonstrated that carcinogenesis in other organs, such as lung, stomach, breast, oral cavity, esophagus, pancreas, prostate, duodenum, and colon, was also inhibited by GTPPs.[6-8] The molecular targets for GTPPs include Ras and activator protein (AP)-1, elements of the mitogen-activated protein kinase (MAPK) signaling pathway.[9] Recently, the properties of GTPPs for anti-inflammatory, antiaging, and wound-healing effects were also explored.[10-12] Evidence generated from basic science laboratories indicated that GTPPs are not only a group of reactive oxygen species (ROS) scavengers that function as antioxidants in the epidermis, but also act as modulators of different gene groups and signal pathways. This review article examines the progress in research on green tea for skin protection and improvement, discusses the possible mechanisms involved in signaling and the various factors regulated by GTPPs, and extends an outlook to future directions in this field. However, epidemiologic and human studies have so far not generated conclusive results, which may be a result

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of multiple factors, such as the conditions used in animal studies versus human green tea consumption, and different bioavailabilities between human beings and rodents.[8,13]

GREEN TEA AND GTPPS

The tea plant (Camellia sinensis) has been cultivated in Asia for thousands of years. Currently, more than two thirds of the world population consumes this popular beverage. However, the majority of the tea consumed in the world is black tea (78%), whereas green tea consumption comprises only 20%.[14] China is the second largest tea producer in the world, but is the largest producer and consumer of green tea. The unique feature of green tea production is that the processing of the tea leaves does not involve any form of fermentation. Freshly picked tea leaves are briefly heated in a pan or by steam without any additives. The brief heating inactivates polyphenol oxidase, thereby preserving the antioxidant activities of the polyphenols. The 4 major polyphenolic catechins present in green tea leaves are (−)-epicatechin (EC), EGC, (−)-EC-3-gallate, and EGCG, which is the most abundant.[7,8,11] The total content of polyphenols in tea leaves varies from approximately 20% to 40%, depending on the subspecies of the plant and geographic location. The polyphenols are readily extracted from green tea leaves by water or organic solvents such as methanol and ethanol.

In contrast, production of black tea involves many more steps, such as withering and fermentation (oxidation). These steps ensure the enzymatic oxidation of the polyphenolic catechins. Oxidation converts these polyphenols to theaflavins and thearubigins, which make the taste and aroma of black tea more appealing to most consumers in the West. Tea consumption (mostly green tea) in China is 330 g/person/annum, which converts to about one cup/person/d (China's Ministry of Agriculture, June 5, 2004). Statistically, green tea–consuming populations in China and Japan have much lower incidences of certain cancers compared with nongreen tea–consuming populations. Comparison of the United States and China reveals glaring facts: the Chinese male population has a much lower incidence of oral cancer, bladder cancer, prostate cancer, and colon cancer. In particular, given the fact that the Chinese population ranks number one in smoking, with 350 million cigarette smokers, more than one fourth of the total world smoking population (China's Ministry of Health, December 2, 2004), the incidence of digestive and urinary tract cancers is still remarkably low. A similarly lower incidence is found in Japan, another green tea–consuming and heavy-smoking population.[15,16] These facts suggest that green tea consumption may protect the epithelial surface from carcinogenesis in human beings.

PIONEERING STUDIES IN PROTECTION OF SKIN CARCINOGENESIS BY GREEN TEA

Skin has the largest epithelial surface of all organs, and skin cancer is the most common type of cancer in the United States according to the CDC.[1] Excluding melanoma, the incidence of basal cell carcinoma and squamous cell carcinoma of skin is estimated to exceed 1 million per year.[17] On the other hand, skin cancers are among the most preventable, because the primary cause of these malignancies is shortwave UV radiation from the sun. GTPPs were first found to prevent skin cancer in a chemical-induced skin-cancer model. During the late 1980s, a group headed by Hasan Mukhtar[18] at Case Western Reserve University (Cleveland, Ohio) applied GTPPs topically on Sencar mice at a dose of 24 mg/mouse for 7 days before exposure to a single dose of 200 nmol of the initiating agent, (+/−)-7 β, 8 α-dihydroxy-9 α,10 α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. This was followed by topical applications twice weekly of the tumor promoter TPA. Results showed that GTPPs had a significant inhibitory effect on tumor induction in this initiation-promotion model.[18] These investigators also tested topical application of GTPPs in a complete skin tumorigenesis protocol using 3-methylcholanthrene on BALB/c mice, and a two-stage skin tumorigenesis protocol using DMBA as the initiating agent and TPA as tumor promoter with Sencar mice. Significant protection by GTPPs against skin tumorigenicity was demonstrated.[19] These findings represent the first topical application of GTPPs for protection from skin cancer and served as a foundation for subsequent studies. A mechanistic study of GTPPs' protective effect followed, which showed that the antioxidant activity of GTPPs could be responsible for the anticarcinogenic potential against TPA and free radicals.[20] It was later found that EGCG was a potent anticarcinogen in a chemically induced cutaneous cancer model. EGCG significantly inhibited binding of ^{3}H-labeled polycyclic aromatic hydrocarbons to epidermal DNA. Topical pretreatment of mice with EGCG resulted in significant reduction in tumor size and number per mouse in a DMBA tumor-initiation model.[21]

The first UVB radiation-induced photocarcinogenesis study using GTPPs as a protection agent was reported 2 years later by Mukhtar and colleagues.[22] Female SKH-1 hairless mice were either fed 0.1% GTPPs or GTPPs were topically applied, followed by exposure to UVB radiation. Both applications afforded photoprotection against UVB. Thus, a new

era of studying skin-cancer prevention by phytochemicals present in a popular beverage had begun.[23]

SUBSEQUENT FINDINGS FROM ANIMAL MODELS

UV protection was the main focus for earlier animal studies of green tea extracts.[24] Using hairless mice, oral consumption or topical application of brewed green tea, green tea extracts, or GTPPs showed significant protection against UV or chemical-induced carcinogenesis. One of the earlier studies used brewed green tea as the sole fluid source for SKH-1 mice during carcinogenesis initiated by either UVB or DMBA and promoted by either TPA or UVB, respectively. Oral consumption of brewed green tea at concentrations similar to human consumption (1.25% and 2.5%) significantly inhibited UVB- or TPA-induced tumorigenesis.[25] This group, led by Conney[26] at Rutgers University, later found that oral administration of decaffeinated green tea possesses similar anticarcinogenic effects. They later showed that green tea oral administration in mice not only inhibited skin tumorigenesis but also reduced fatty tissues in the dermis.[27] Mechanistically, oral administration of GTPPs resulted in decreased UVB-induced ornithine decarboxylase and cyclooxygenase (COX) activities.[28] Oral administration or intraperitoneal injection of GTPPs could achieve similar effects to inhibit the growth of UV-induced skin papillomas[29] or TPA-induced COX2 in rodent models.[30]

In models of topical application, GTPPs inhibited DMBA-initiated, TPA-induced carcinogenesis and benzo[a]pyrene- and TPA-induced tumor initiation in CD-1 mice.[31,32] Topical application of GTPPs reduced TPA-induced inflammation, ornithine decarboxylase activity, hyperplasia, and hydrogen peroxide (H_2O_2) formation, suggesting GTPPs serve as both an antioxidant and a regulator of enzymatic activities.[31] TPA-induced elevation of COX and lipoxygenase were significantly inhibited by topical application of GTPPs to Sencar mice,[31] and EGCG was found to be the most effective agent tested in reducing epidermal ornithine decarboxylase activity induced by chemical tumor promoters.[33]

It appears that GTPPs provide a shield effect against photocarcinogen-induced oxidative stress. Topical application of GTPPs (5 mg/animal) or EGCG (1 mg/cm^2 of skin) before UVB exposure in SKH-1 hairless mice significantly prevented UVB-induced depletion of the antioxidant enzymes glutathione peroxidase, catalase, and glutathione; inhibited UVB-induced oxidation as measured by lipid peroxidation and protein oxidation; and inhibited UVB activation of MAPK family members extracellular signal-regulated kinase (ERK) 1 and 2, c-jun N-terminal kinase (JNK), and p38.[34,35] GTPPs can be rapidly metabolized by reacting with ROS, providing the first line of defense against ROS in vivo, and they also inhibit pro-oxidant enzymes such as nitric oxide synthase, lipoxygenases, COX, and xanthine oxidase, which provide the second line of defense against free radicals, especially H_2O_2.[36]

An interesting study used SKH-1 hairless mice, which are at high risk for skin cancers, to mimic the frequent exposure of UVB as a photocarcinogen. The mice were exposed to UVB twice a week for 20 weeks before daily topical application of EGCG (6.5 μmol/L) for another 18 weeks. The results showed that EGCG decreased the number of nonmalignant and malignant tumors per mouse by 55% and 66%, respectively, suggesting that the effect of EGCG was not just caused by a sunscreen/antioxidant effect. In fact, apoptotic activities were significantly increased in tumor cells but not in normal cells.[37]

Oral versus topical administration of GTPPs

The animal studies discussed above suggest that oral administration of GTPPs can provide skin protection in rodents. However, similar studies in human beings did not achieve such effects, presumably because the human dermis provides a stronger barrier to absorption from the vasculature.[38] Conversely, rodents appear to possess a weaker barrier, in that topical application of EGCG at a high concentration (10% in hydrophilic ointment *United States Pharmacopeia*) resulted in toxicity in SKH-1 mice and formation of erythema and papular lesions within days,[39] but human skin showed no side effects.[40] Therefore, the effects of topical application of GTPPs, rather than oral administration, may have a greater clinical significance in human beings.

EVIDENCE IN HUMAN STUDIES

Compared with animal studies, evaluation of GTPPs in human skin has been very limited. EGCG effectively penetrates the dermal barrier in mice but not in human beings.[38] In a human biopsy study, green tea extracts and individual polyphenols were applied topically for 30 minutes before a 2-minute exposure to UV irradiation, and the skin samples were analyzed by immunohistochemistry. The topical application of these agents caused a dose-dependent inhibition of the erythema response induced by UV radiation. Green tea extracts reduced the number of sunburn cells and DNA damage. Among the GTPPs, EGCG and (−)-EC-3-gallate (the polyphenols that possess a gallate group) were effective, whereas EGC and EC (the polyphenols

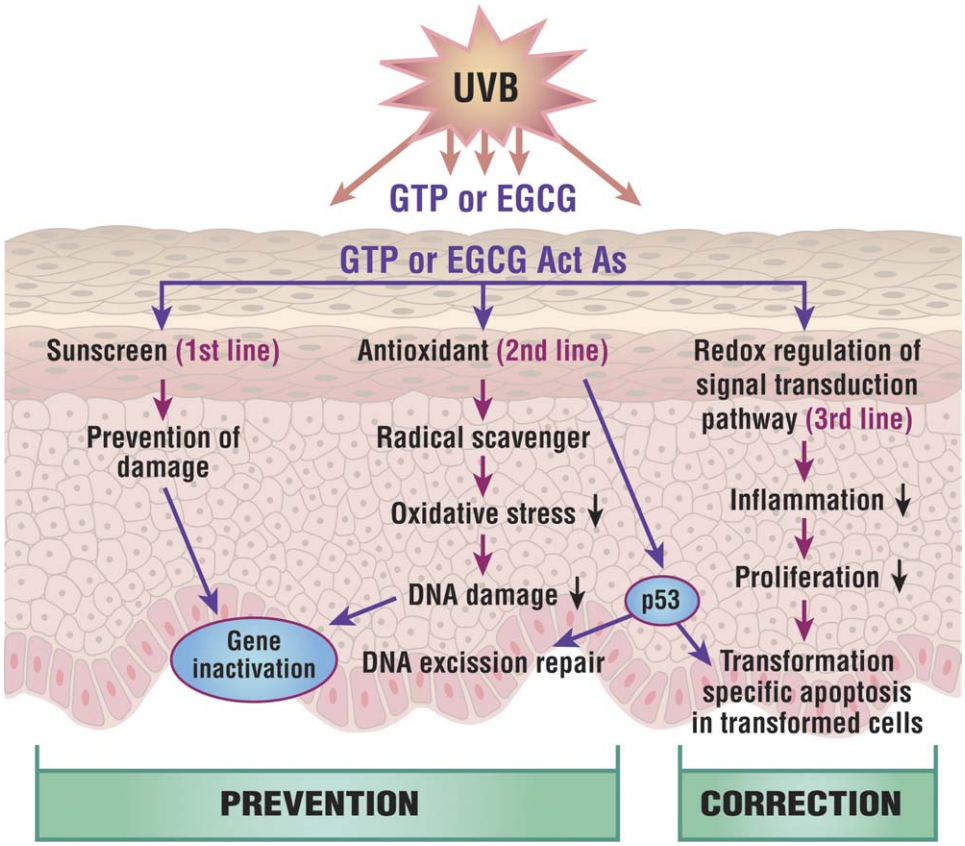

Fig 1. Schematic illustration of properties of green tea in photoprotection against UV irradiation, summarized from previously published data.[4,35,41,52,59,69,70] Only epidermis is shown. *EGCG*, (−)- Epigallocatechin-3-gallate; *GTP*, green tea polyphenol.

that lack a gallate group) were ineffective.[41] When EGCG at 3 mg/2.5 cm^2 was applied before UVB exposure, UVB-induced erythema and UVB-induced infiltration of leukocytes were reduced.[42] Thus, GTPPs could be applied for photoprevention in human beings. Fig 1 summarized the major protective effects of GTPPs against photocarcinogenesis.

A combination of psoralen and UVA (PUVA) therapy has been used for treatment of certain skin diseases, such as psoriasis, to reduce toxicity that is caused by systemic drugs.[43] However, prolonged treatment with PUVA increases the risk of skin cancer, especially squamous cell carcinoma.[44] A study using several in vitro and in vivo models, including human beings, found that 30-minute topical application of 0.2 mg/cm^2 green tea extract almost completely inhibited PUVA-induced erythema; the same extract also inhibited DNA damage caused by PUVA, suggesting that GTPPs protect the epidermal keratinocytes from PUVA therapy–induced carcinogenesis.[45,46] Another interesting finding was that green tea may help to protect hair follicles from γ-ray–induced apoptosis.[47] A number of human trials using GTPPs as chemopreventive agents against skin carcinogenesis are currently under way, targeting COX2 and the MAPK signal pathways. The goal of these trials is to develop formulations of agents such as GTPPs in sunscreen and other protective skin applications for use against UVB-induced carcinogenesis.[48,49]

MECHANISMS OF GTPP-INDUCED EFFECTS

Differential effect in normal versus malignant cells

Because GTPPs and EGCG were found to selectively induce apoptosis in tumor cells but not normal human epidermal keratinocyte (NHEK), in vitro studies on the molecular mechanisms for the chemopreventive properties of GTPPs were initiated.[50] Compared with epidermoid carcinoma cells, NHEKs showed less sensitivity to EGCG-induced inhibition of nuclear factor (NF) κB, a prosurvival transcription factor often up-regulated in tumor cells, suggesting tumor cells are more susceptible to EGCG-induced apoptosis and growth arrest.[51] On the other hand, pretreatment of NHEKs with EGCG suppressed UVB-induced activation of NF κB in a

dose- and time-dependent manner.[52] Similarly, EGCG inhibited TPA-induced NF κB in a mouse cell line model.[53] It appears that this observation is contradictory to the prosurvival effect of EGCG in NHEKs. But it may be interpreted as the activation of a terminal differentiation pathway, which involves a caspase 14-mediated planned cell death. Therefore, inhibition of the prosurvival transcription factor NF κB might be required.

UVB protection

In human keratinocytes, EGCG inhibited UVB-induced AP-1 expression, suggesting that AP-1, a group of downstream transcription factors in the MAPK pathway, including c-Fos and c-Jun, could be a target for EGCG function.[54] MAPKs are important signal transduction proteins that can be activated through serial phosphorylation cascades and regulate gene expression in response to exogenous stimuli. Depending on the stimuli, the subsequent cellular responses induced by the downstream factors of the MAPK pathway determine the fate of a cell through the activation of either antiapoptotic or proapoptotic genes. In general, among the MAPK pathways involving ERK, JNK, and p38, ERK is activated by mitogens and growth factors, and p38 and JNK are activated by stress stimuli such as UV irradiation or oxidants, resulting in apoptosis.[55]

In a keratinocyte cell line HaCaT, UVB-induced c-Fos expression was specifically inhibited by EGCG at both the messenger RNA and protein levels. UVB-induced activation of MAPK p38, the upstream factor of c-Fos, was also inhibited by EGCG. However, c-Jun activation was not modulated by EGCG, nor was its upstream activator, JNK.[56] This might be a result of the induction of p57/KIP2, a cyclin-dependent kinase inhibitor, which binds and inhibits the activity of JNK.[12,57,58] A more comprehensive study demonstrated that pretreatment of NHEKs with EGCG inhibited UVB-induced intracellular H_2O_2, and UV-induced phosphorylation (activation) of ERK 1 and 2, JNK, and p38.[59] In addition, UVB-induced degradation and phosphorylation of IκBα and activation of IKKα were inhibited by pretreatment of EGCG for 24 hours, therefore, preventing the nuclear localization of NF κB.[36] These effects could be ascribed to EGCG's regulatory effects on the MAPK pathway and its antioxidant activity because EGCG is able to scavenge ROS and bring ROS to background levels in NHEKs.[60]

UVA protection

UVA is another type of shortwave UV radiation. EGC inhibited UVA-activated COX, a stress-responding gene product, in epidermoid keratinocytes, which suggested that the effect of this GTPP may involve direct effects on signal transduction, in addition to its antioxidant activity.[61] Thus, GTPPs appear to regulate stress-responding pathways, specifically the MAPK pathways. With respect to UVA-induced ROS, GTPPs were effective in reducing the toxicity caused by UVA-induced ROS in primary rat epidermal keratinocytes, with inhibition of the UVA-activated release of the plasma protein lactate dehydrogenase and up-regulation of UVA-suppressed glutathione peroxidase activity.[62] In healthy primary rat epidermal keratinocytes, GTPPs were able to inhibit lactate dehydrogenase release and increase glutathione peroxidase activity at a concentration range of 0.05% to 0.1%. When cell growth and apoptosis were measured, 0.01% to 0.1% GTPPs stimulated cell proliferation and inhibited apoptosis, suggesting that GTPPs may potentially benefit skin healing by promoting keratinocyte proliferation.[63]

As a multifunctional agent

UVB increases the levels of intracellular H_2O_2 and activates the MAPK pathway in NHEKs, and EGCG inhibits them both. Pretreatment of 20 μmol/L EGCG reduced UVB-elevated H_2O_2 and phosphorylation of ERK 1 and 2, JNK, and p38 by more than 50%.[60] However, in addition to the photoprotection effects from EGCG, the MAPK signal transduction pathway regulated by EGCG in NHEKs appears to be associated with other functions, because EGCG modulates this pathway in NHEKs in the absence of stress inducers. In contrast to EGCG-treated tumor cell lines, immortalized epithelial cell lines, or UV-treated NHEKs (where EGCG/GTPP inhibited MAPK activation), EGCG increased the activities of certain elements in the MAPK pathway in exponentially growing NHEKs. Specific regulation by EGCG of an AP-1-activated gene, involucrin, was reported.[64] Human involucrin is a marker for NHEK intermediate to late differentiation, and its promoter can be activated by AP-1 elements. EGCG induced the phosphorylation of Ras, MEKK1, MEK3, and p38δ (an isoform of p38) of the MAPK signal transduction pathway and induced AP-1 transcription factors c-Jun and c-Fos, and other AP-1 family members, Fra-1, Fra-2, FosB, JunB, and JunD (JNK activation was not measured in this study). In addition, cornification of the NHEKs was observed.[64] Thus, whether EGCG is involved in NHEK differentiation became an interesting issue. We reported that EGCG selectively induced p57/KIP2, the only KIP/CIP family member of the cyclin-dependent kinase inhibitors involved in development. The induction of p57 by EGCG was only found in NHEKs but not in epithelium-derived tumor cells.[57] p57 is linked with the MAPK pathway

Table I. Summary of multiple effects of green tea polyphenols and the cellular/molecular responses induced in the epidermal systems

Green tea-induced effects	Cellular/molecular responses	References
UV protection	Inhibition of tumorigenesis; inhibition of UV-induced MAPK activation; inhibition of UV-induced AP-1 activation; inhibition of UVA-induced LDH; up-regulation of UVA-suppressed GSH-Px; inhibition of UVB-induced infiltration of macrophages and neutrophils	5, 11, 22-26, 29, 34, 35, 37, 41, 48, 49, 54-63, 71
Antioxidant	Elimination of reactive oxygen species; stabilization of GSH-Px, catalase, and glutathione; inhibition of nitric oxide synthase, lipoxigenase, COX and xanthine oxidase; inhibition of lipid peroxidase	11, 31, 34-36, 60, 68, 69, 75
Anti-inflammation	Inhibition of ODC, COX, lipoxigenase; inhibition of IL-1, IL-8, IL-10 and IL-12 release; inhibition of UVB-induced infiltration of macrophages and neutrophils	28, 31, 33, 70-73
Acceleration of keratinocyte differentiation and wound healing	Induction of p57, filaggrin, keratins, involucrin and transglutaminase activity; induction of caspase 14	12, 31, 57, 64, 88
Anticarcinogen	Inhibition of tumorigenesis; inhibition of carcinogen-DNA binding	18-21, 30-33, 74
Protection of PUVA-induced carcinogenesis	Inhibition of erythema and DNA damage	45, 46
Protection of hair follicles from radiation	Inhibition of radiation-induced apoptosis	47

AP, Activator protein; *COX*, cyclonoxygenase; *GSH-Px*, glutathione peroxidase; *LDH*, lactate dehydrogenase; *MAPK*, mitogen-activated protein kinase; *PUVA*, psoralen plus ultraviolet A light.

by negatively regulating JNK activation through direct binding.[58,65] EGCG-induced p57 may modulate the activity of JNK to prevent caspase 3-mediated apoptosis, because persistent activation of JNK leads to apoptosis.[65] Indeed, cells that lack p57 induction underwent caspase 3-dependent apoptosis in response to EGCG.[66] It was later found that EGCG-induced p57 was associated with rapid terminal differentiation marked by increased expression of keratins and filaggrin, and activated transglutaminase activity within 24 hours in exponentially growing NHEKs. On the other hand, aged NHEKs (25 days postconfluence) showed new DNA synthesis, suggesting a potential role in aged keratinocyte proliferation by EGCG.[12] In a human study, topical application of 10% EGCG stimulated NHEK proliferation and increased skin thickness in elderly men, which further confirmed the possible stimulatory effects of EGCG in the aged epidermis.[13]

In summary, as powerful antioxidants, GTPPs scavenge ROS produced by UV or other sources (ie, carcinogenenic chemicals and autosynthesis mechanisms). As active nutriceuticals, GTPPs are able to: (1) modulate elements in the Ras-MAPK signal pathway, including ERK, JNK, and AP-1; (2) inhibit COX2, phase I and phase II enzymes; (3) inhibit inflammation-related responses, such as IL-1, IL-10, and IL-12 release; (4) activate a novel terminal differentiation pathway associated with p57 and caspase 14 expression, and accelerate skin barrier formation; (5) suppress the caspase 3-mediated apoptosis pathway and activate caspase 14-mediated planned cell death pathway in normal cells; and (6) stimulate proapoptotic genes such as p53 and p21, and suppress the activity of the prosurvival transcription factor NF κB in tumor cells.

Table I lists the major effects of GTPPs found on normal epidermal systems.

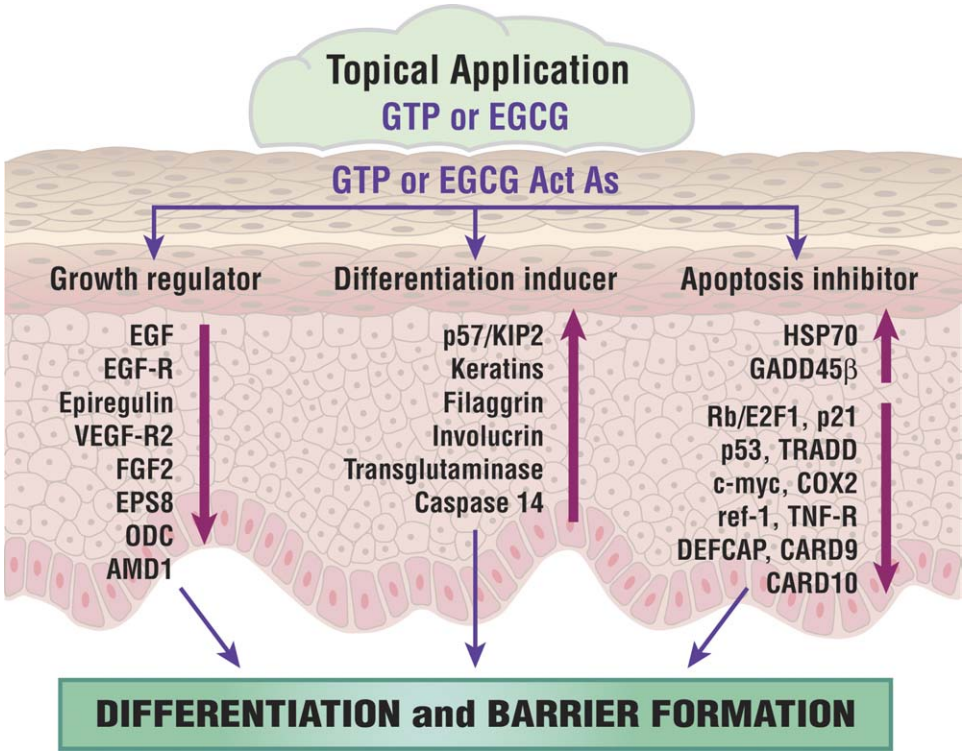

Fig 2. Expression of green tea targeted genes in response to (−)- epigallocatechin-3-gallate (*EGCG*) exposure in human epidermal keratinocytes, based on previously published findings.[12,57,67,90] Certain cell growth regulating genes are inhibited, major markers for keratinocyte differentiation are up-regulated, whereas many genes coded for proapoptosis factors are suppressed. Only epidermis is shown. AMD1, S-adenosylmethionine decarboxylase 1; CARD, caspase recruitment domain; COX 2, cyclooxygenase-2; EGF, epidermal growth factor; EGF-R, epidermal growth factor—receptor; EPS8, a substrate for the epidermal growth factor receptor kinase; FGF2, fibroblast growth factor 2; GADD45β, growth arrest and DNA damage 45β; GTP, green tea polyphenol; HSP70, heat-shock protein 70; ODC, ornithine decarboxylase; Rb, retinoblastoma; TNF-R, tumor necrosis factor receptor; TRADD, tumor necrosis factor receptor 1 (TNFR1)—associated death domain protein; VEGF-R2, vascular epithelial growth factor-receptor 2.

POTENTIAL APPLICATIONS FOR ANTIPHOTOAGING

Green tea and EGCG effectively scavenged UV-induced reactive ROS, therefore, protecting DNA from UV-induced damage.[67,69] In addition, EGCG at 3 mg/animal prevented UVB-induced IL-10 production in the skin, indicating the ability of modulating the immune response by EGCG.[70] A cell surface marker, CD11b, for activated macrophages and neutrophils was decreased by EGCG in animals treated with UVB, suggesting an inhibition of UVB-induced infiltration of these cells associated with immune suppression.[71] In addition, tumor necrosis factor α-induced IL-8 release was dose-dependently inhibited by EGCG in cultured NHEKs, whereas vascular epithelial growth factor was stimulated.[72] Another study demonstrated that TPA-induced IL-1 expression was significantly inhibited by GTPPs.[73] In mouse epidermal cells, EGCG inhibited TPA-induced NF κB activation by blocking phosphorylation of IκBα (inhibitor of NF κB) at Ser32 and inhibited the DNA binding of NF κB.[74] The protective properties of GTPPs against UV-irradiation possess potential value for antiaging purposes, especially for the prevention of photoaging, which causes roughness and sagginess of the skin. In a study using a combination of guinea pigs, hairless mice, and human dermal fibroblast cultures, EGCG was found to reduce the UVB-induced lipid peroxide level by 3-fold, prevented UVA-induced skin damage (roughness and sagginess), and inhibited the expression of collagenase in cultured human epidermal fibroblasts and the promoter-binding activities of AP-1 and NF κB.[75] Oxidative damage induced by UVB may also cause modifications of proteins, eg, collagen cross-linking and formation of carbonyl derivatives, which

can be measured by a fluorescence assay and the reduction reaction method. A study using C57BL/6 mice found that collagen cross-linking could be reduced by a green tea extract at 10 months of age.[75]

POTENTIAL BENEFITS FOR OTHER SKIN DISORDERS AND WOUND HEALING

In addition to anticancer and photoprotection potentials, green tea may provide alternative treatment for other skin disorders. In the epidermis, keratinocytes exist in various stages of differentiation.[76,77] Abnormalities in any of the programmed differentiation events may lead to epidermal disorders, such as psoriasis and skin cancer. However, biologic events that enable basal cells (stem cells) to proliferate, differentiate, and commit planned cell death[78] are still poorly understood.[64] Keratinocyte differentiation can be accelerated by prodifferentiation agents such as extracellular calcium and retinoids, but cancer cells derived from the epidermal epithelium lose the ability to respond to the prodifferentiation agents.[79] Abnormal, or lack of, differentiation also can be found in other skin disorders, such as psoriasis, actinic keratosis, cherry angiomas, Bateman's purpura, chondrodermatitis nodularis helicis, seborrehic keratosis, and rosacea. In these skin disorders, the skin barrier is often disrupted.[80]

A caspase family member, caspase 14, was identified in 1998 from murine tissues, and found only in epithelial tissues, especially in the differentiating epidermis.[81-83] Unlike the other caspases, caspase 14 is not involved in the well-documented apoptotic caspase cascade, but is associated with terminal differentiation of NHEKs and skin barrier formation.[57,79,84] Transcriptional activation of caspase 14 was found during stratum corneum formation.[85] Caspase 14 expression was diminished on inhibition of cell differentiation.[86] Therefore, caspase 14 is believed to facilitate epidermal differentiation, possibly activating planned cell death and cornification of the epidermal keratinocytes to form the skin barrier. In contrast, in pathologic conditions such as psoriasis, in which cornification is altered, the normal expression pattern of caspase 14 is absent.[87]

In our recent studies, we found that EGCG activates a coordinated expression of p57 and caspase 14 in NHEKs, which facilitates terminal differentiation in these cells. In contrast, a squamous cell carcinoma cell line OSC2 and salivary cancer cell line HSG, and human psoriatic keratinocytes, only exhibit basal levels of caspase 14. This study led to our finding that EGCG is able to induce caspase 14 expression in exponentially growing NHEKs within 24 hours, subsequent to p57 induction,[88] but human psoriatic tissue lacks nuclear translocation of caspase 14.[89] Psoriasis has been considered to be an immunologic phenomenon. However, phenotypically it manifests as an epithelial disorder with abnormal morphologic and functional features. In this regard, our observation that psoriatic keratinocytes lack the nuclear entry of caspase 14 might be linked to the failure of cornification and appropriate barrier formation in psoriatic keratinocytes. Thus, induction of caspase 14 expression and nuclear localization by green tea may promote differentiation and skin barrier formation, which may lead to new treatments for skin disorders that lack normal differentiation. The green tea–regulated gene expressions in accelerating epidermal keratinocyte differentiation are profiled in Fig 2. Combined with other properties of green tea, such as stimulation of aged keratinocytes and inhibition of apoptosis, green tea may assist the healing process in the epidermis when applied topically.

FUTURE PERSPECTIVE

Although the majority of the evidence shows remarkable benefits of antioxidant, anticancer, antiaging, and anti-inflammation effects from green tea constituents, standard delivery systems for topical application of GTPPs have not been established. This is partially because of the nature of these highly reactive compounds, which are easily oxidized in the environment and gradually lose their activities if not used immediately after preparation. Therefore, the primary goal for a topical formulation is to maintain the stability of these antioxidants. A preformulation study of EGCG concluded that multiple factors should be considered with regard to the stability issue and that EGCG is rapidly degraded if it is in an aqueous formulation. Thus, although potential synergistic effects must be evaluated, simple mixing of EGCG or GTPPs with other antioxidants may not increase stability.[90] An earlier study that tested 10% EGCG in hydrophilic ointment *United States Pharmacopeia* suggested that addition of 0.1% butylated hydroxytoluene to this formulation boosted the stability significantly.[38] These types of formulations, with combined active compounds, are still to be evaluated in human trials. Therefore, the stability issue of GTPPs for topical application remains to be resolved.

The second challenge is the epidermal penetration. With the exceptions of abnormal conditions such as traumatic open wounds, infections, or pathologic lesions, human skin is a waterproof barrier protected by multilayers of cornified keratinocytes. The hydrophilic GTPPs in an aqueous phase of a formulation, regardless of stability, may have to rely on high concentration to penetrate this barrier

and achieve effectiveness, as previously shown.[13] However, recent tests using in vitro delivery models demonstrated that solutions saturated with green tea extract, or adhesive patches with more than 1 mg/cm^2 green tea extract failed to deliver the polyphenols, after a 24-hour period, in a concentration higher than the maximum serum concentration (<10 μmol/L).[91] On the other hand, a high concentration of EGCG, such as 10%, has not been tested for long-term toxicity. In addition, high concentrations of GTPPs or EGCG in a formulation would considerably increase the cost. Therefore, approaches other than simply increasing the concentration of GTPPs or EGCG may be required as alternative strategies. For example, changing the physical properties of GTPPs by molecular modification to increase the stability and improve skin penetration may be an option. Taken together, the 3 major challenges, ie, stability, penetration, and cost, need to be addressed to effectively bring the benefits of green tea to the human skin.

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