# Green tea polyphenol induces caspase 14 in epidermal keratinocytes via MAPK pathways and reduces psoriasiform lesions in the flaky skin mouse model

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**Abstract:** Psoriasiform lesions are characterized by hyperproliferation and aberrant differentiation of epidermal keratinocytes, accompanied by inflammation, leading to a disrupted skin barrier with an abnormal stratum corneum. The expression and proteolytic processing of caspase 14, a member of the caspase family which is associated with epithelial cell differentiation, planned cell death, and barrier formation, is altered in psoriatic epidermis. We recently reported that human psoriatic tissues lack normal expression of caspase 14 [*J Dermatol Sci* **37** (2005) 61], and caspase 14 is induced by EGCG, a green tea polyphenol (GTP), in exponentially growing normal human epidermal keratinocytes (NHEK) [*J Pharmacol Exp Ther* **315** (2005) 805]. This suggests that GTPs may have beneficial effects on psoriasiform lesions. The current study aimed to determine whether MAPK pathways are required for GTP-induced caspase 14 expression in NHEK and if GTPs can modulate the expression of pathological markers in the psoriasiform lesions that develop in the flaky skin mouse. The results indicate that the p38 and JNK MAPK pathways are required for EGCG-induced expression of caspase 14 in NHEK. Importantly, topical application of 0.5% GTPs significantly reduced the symptoms of epidermal pathology in the flaky skin mice, associated with efficient caspase 14 processing and reduction in proliferating cell nuclear antigen levels. This suggests that GTP-activated pathways may be potential targets for novel therapeutic approaches to the treatment of some psoriasiform skin disorders.

**Key words:** caspase 14 – EGCG – epidermal – green tea – keratinocyte polyphenols – psoriasiform lesions

## Introduction

Psoriasiform lesions, such as those in psoriasis, involve hyperproliferation and aberrant differentiation of epidermal keratinocytes, and inflammation, leading to a disrupted barrier characterized by an abnormal stratum corneum. The pathogenesis of psoriasis, which has both epidermal and immunological components, is still under investigation. The most common current treatments (e.g. phototherapies, potent topical steroids, oral retinoids, immunosuppressive agents such as methotrexate or cyclosporine) are often associated with untoward side effects (1,2). Clearly, more work is required to identify treatments that are safe, effective, convenient to administer, and reasonably priced.

Caspase 14 is expressed only in epithelia, particularly tissues involve in barrier formation such as retinal pigment epithelium, and especially in the differentiating epidermis (3–7). Unlike other caspases, caspase 14 is not involved in the pro-apoptotic caspase cascade, but is associated with terminal differentiation of NHEK, cornification and nuclear destruction of the NHEK, and with barrier formation (8–11). In the epidermis, induction of caspase 14 at the transcriptional level was noted during stratum corneum formation (6). Conversely, caspase 14 expression was diminished following inhibition of cell differentiation by retinoic acid (12). Collectively, caspase 14 is believed to facilitate epidermal differentiation, possibly activating planned cell death and cornification of the epidermis to form the skin

barrier (11,13). In contrast, in pathological conditions such as psoriasis, in which cornification is interrupted, the normal expression and processing patterns of caspase 14 are defective (8,10). The signal pathway(s) leading to caspase 14 expression and activation (by cleavage of the 28 kDa form into 17 and 14 kDa subunits), and the proteolytic enzyme(s) responsible for caspase 14 processing, are unknown, as are the endogenous substrates for this enzyme.

Green tea polyphenols (GTPs), also referred to as catechins, are present in the leaves of the tea plant (*Camellia sinensis*). GTPs are known antioxidants that also possess anticancer activities. The four major polyphenols present in green tea leaves are (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocatechin-3-gallate (EGCG). EGCG is the most abundant and considered the most potent GTP (13,14). Uniquely, GTPs selectively induce growth arrest and apoptosis in many types of tumor cells while protecting certain normal epithelial cells (14–19). We found previously that GTPs or EGCG, at a topically achievable range, selectively induced caspase 3-dependent apoptosis in tumor cells, while normal human epidermal keratinocytes (NHEK) showed elevated expression of p57/KIP2 and underwent terminal differentiation in response to EGCG, without activation of caspase 3 (20–23). Results from our recent study demonstrated an inhibitory effect of EGCG on autoantigen expression, and are consistent with a green tea-activated differentiation pathway (24,25).

p57, a cyclin-dependent kinase inhibitor, is a suspected inducer of caspase 14 (26). In a previous study, we investigated the relationship between EGCG and caspase 14 expression in an *in vitro* tumor versus normal cell system: NHEK, OSC2 (an oral squamous cell carcinoma line), and stably-transfected subclones derived from OSC2 cells that overexpress p57/KIP2. The results demonstrated that EGCG coordinately activates the expression of p57 and caspase 14 in NHEK, which facilitates terminal differentiation in these cells (26). We also found that human psoriatic tissue lacks significant nuclear localization of caspase 14 (26,27). This may explain the failure of cornification and appropriate barrier formation in psoriatic epidermis. Thus, the potential use of green tea constituents as an alternative non-toxic preventive agent or therapeutic component to treat psoriasiform lesions warrants exploration.

It has been reported that knockout mice for the downstream transcription factors of the MAPK pathways, AP-1 components c-Jun and JunB, developed psoriasiform lesions that resemble human psoriasis, presumably independent from changes in T- and B-cell immunity (28). As the inducible knockout was specific to the epidermis, and EGCG is known to regulate the MAPK pathways in many cell types (25,29,30), these findings suggest that EGCG-induced caspase 14 and epidermal keratinocyte terminal differentiation may rely on modulation of MAPK pathways, especially the p38 MAPK pathway, upstream of AP-1 components. The current study examined caspase 14 expression in various cell types, determined the MAPK pathways that are required for induction of caspase 14 expression by GTPs, and explored the use of GTPs to modulate the psoriasiform lesions in flaky skin mice.

## Materials and methods

### Cell lines
Cell cultures of OSC2 (21) and HSG (a human salivary gland cancer cell line, American Type Culture Collection) were previously described (25). A431, a human epidermoid cancer cell line, was purchased from ATCC, and cultured in Dulbecco's modified Eagle's medium. Pooled NHEK were purchased from Cambrex (East Rutherford, NJ, USA) and cultured in the specific growth media (KGM-2) provided by the manufacturer.

### Chemicals and antibodies
EGCG was purchased from Sigma-Aldrich (St Louis, MO, USA), and dissolved in cell culture media immediately prior to use. The green tea extract (GTE) with 90% GTPs and containing 40% EGCG, 13% ECG, 7.3% EGC, 3.2% EC, and 2.7% caffeine was provided by Zhejinag Cereals, Oils & Foodstuffs Imp/Exp Co., Ltd, Hangzhou, China. Pharmacological inhibitors for p38 (SB 203580), JNK (Sp600125) and MEK (PD 98059) were supplied by EMD Bioscience, Inc. (San Diego, CA, USA). The anti-caspase 14, anti-proliferating cell nuclear antigen (PCNA) and anti-human actin (I-19) antibodies were obtained from Santa Cruz Biotechnology, Santa Cruz, CA, USA (H-99).

### Animal treatment
Female homozygous flaky skin mice were purchased from Jackson Laboratories at the age of 3 weeks. Six mice were randomly assigned to two groups and fed *ad libitum*. One group (three mice) was bathed with warm water (37°C) 5 days/week. The other group was bathed with freshly prepared warm water containing 0.5% GTE (37°C). At the end of week 13 of age the animals were killed and skin samples were collected. One portion of the skin samples was fixed and either stained using a standard haematoxylin and eosin (H&E) method, or immunostained with antibodies against caspase 14 or PCNA; the other portion of the skin samples was frozen at −80°C prior to Western analyses.

### Immunohistochemistry
The skin samples from mice were fixed in 10% neutral-buffered formalin, paraffin embedded, sectioned at 5 μm, and either stained with H&E by routine methods as previously

described (31), or immunostained with rabbit anti-caspase 14 antibody (at 1:50 dilution), or anti-PCNA antibody (at 1:100 dilution). Tissues processed with normal rabbit serum in place of the primary antibody served as the negative controls. The slides processed for immunohistochemistry were photographed using an Olympus BX40 microscope with image capturing capabilities.

## Western analyses

The method for Western analyses was described previously (26). For skin tissue sample preparation, frozen skin samples were ground in liquid nitrogen and homogenized in lysis buffer (1% SDS, 1.0 mM sodium orthovanadate, 10 mM Tris–HCl, pH 7.4, and 2 mM EDTA). Lysates (cell, 30 $\mu$g protein; skin, 50 $\mu$g protein per lane) were separated on a 15 % SDS polyacrylamide gel prior to Western blotting with different antibodies (caspase 14 1:1000; actin 1:2000; PCNA 1:1000).

## Results

### Caspase 14 expression in tumor cell lines and NHEK

To compare caspase 14 expression in various tumor cell lines to NHEK, we determined caspase 14 expression by Western blotting in OSC2 (a human oral carcinoma cell line), A431 (a human epidermoid cancer cell line), HSG (a human salivary gland cancer cell line) cells, and NHEK with or without EGCG exposure. The results indicated that caspase 14 protein was not expressed in A431 or HSG cell lines, while OSC2 cells exhibited a low basal level of caspase 14. When the cells were exposed to 50 $\mu$M EGCG (a physiological level) for 24 h, only NHEK responded by expressing high levels of caspase 14 (Fig. 1a).

### Inhibition of p38 and MEK blocks EGCG-induced caspase 14 expression

To investigate which MAPK pathways are essential for EGCG-induced expression of caspase 14 and p57, NHEK were treated with an inhibitor of p38, JNK or MEK for 30 min prior to exposure to 50 $\mu$M EGCG for 24 h. Western analyses demonstrated that while p57 expression is associated only with the activation of the p38 MAPK pathway, caspase 14 expression utilizes both p38 and JNK pathways. Inhibition of p38 suppressed both p57 and caspase 14 expression in NHEK, while inhibition of JNK suppressed caspase 14 expression, but not p57 (Fig. 1b). The MEK inhibitor had no effect on the expression of either protein.

### Topical application of GTE reduces pathological markers of psoriasiform lesions

The flaky skin mouse is a genetic model for psoriasiform lesions (32,33). At the beginning of week 11, all three
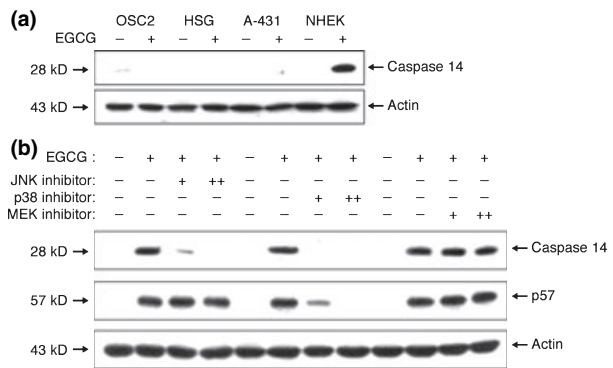


**Figure 1.** (a) Western blot of cell lysates from exponentially growing OSC2, HSG, A431 and NHEK cells exposed to medium with or without 50 $\mu$M EGCG for 24 h. Actin levels are presented as internal loading controls. EGCG specifically induced caspase 14 expression in NHEK but not in any tumor cell line. (b) A p38 inhibitor blocked EGCG-induced caspase 14 and p57 expression. NHEK cells were treated with 50 $\mu$M EGCG for 24 h with or without specific inhibitors of p38, JNK or MEK. Induction of p57 by EGCG was blocked specifically with the p38 inhibitor, but not with the JNK or MEK inhibitor, while caspase 14 expression was blocked by both the p38 and JNK inhibitors (concentration of inhibitors was: + 10 $\mu$M, ++ 30 $\mu$M, added to the cell culture medium 30 min prior to EGCG exposure).

animals in the water-treated group had developed skin lesions but none of the three animals in the water-GTE-treated group had developed skin lesions (data not shown). The GTE-treated animals only began to exhibit visible skin lesions at week 11.5 of age, and these lesions remained moderate until week 13. At the end of week 13, the animals were killed and skin samples were collected. Skin samples were fixed and stained using a standard haematoxylin and eosin (H&E) method. As shown in Fig. 2a, water-treated skin exhibited extensive psoriasiform lesions, characterized by marked hyperkeratosis, mild parakeratosis, modest acanthosis, elongated rete ridges, and modest dermal inflammation, consistent with modest psoriatic-like dermatitis. The water-GTE-treated skin exhibited only slight hyperkeratosis, no parakeratosis, mild acanthosis, no elongated rete ridges, and mild dermal inflammation (Fig. 2b). That is, the skin condition of the GTE-treated mice was significantly improved in comparison with the control mice ($P = 0.025$, one-tailed Mann–Whitney $U$-test for small samples, $n = 3$ per group).

Immunostaining of skin samples from water-treated or water-GTE-treated flaky skin mice with a caspase 14 antibody revealed nuclear localization of caspase 14 at the junction of the granular and cornified layers in water-GTE-treated skin (Fig. 3b, arrows) but not in the water-treated skin (Fig. 3a). Western blot analysis (Fig. 4) showed that caspase 14 was present in the water-treated skin sample predominantly as the catalytically inactive 30 kDa form (equivalent to the human 28 kDa form), but the water-
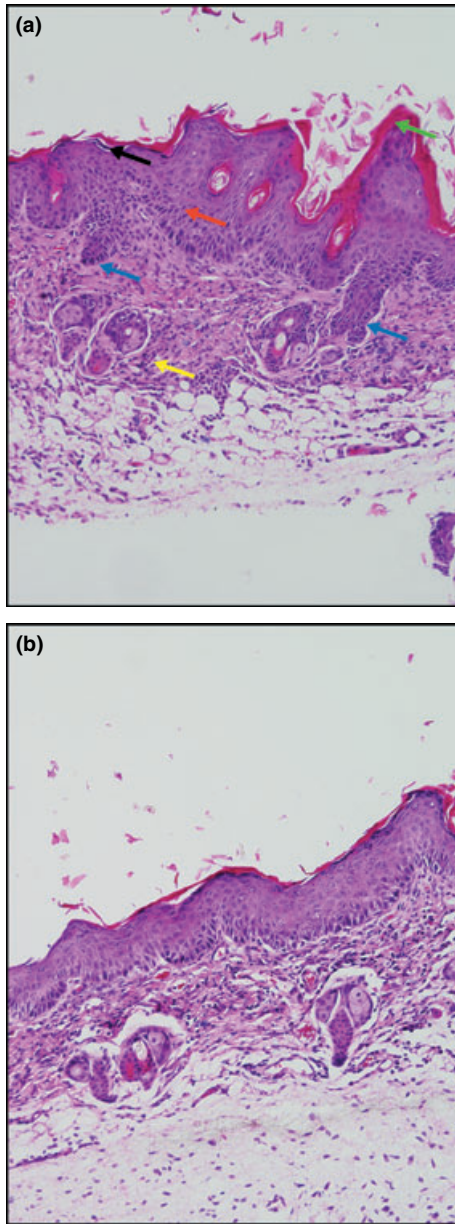
**Figure 2.** Flaky skin mouse (*fsn/fsn*) skin samples stained by a standard H&E method. Animals were treated with a daily water bath or a water-GTE bath starting at an age of 3 weeks. Skin samples were collected at age 13 weeks. (a) The water-treated skin section shows marked hyperkeratosis (green arrow), mild parakeratosis (black arrow), modest acanthosis (red arrow), elongated rete ridges (blue arrows), and modest dermal inflammation (yellow arrow), consistent with psoriatic-like dermatitis. (b) The water-GTE-treated skin section shows slight hyperkeratosis, no parakeratosis, mild acanthosis, no elongated rete ridges, and mild dermal inflammation (100× magnification).

GTE-treated skin showed significantly higher levels of the catalytically active 17 kDa subunit (Fig. 4). As the 17 kDa subunit is associated with the cornified layer, and human psoriatic plaques have predominantly the 28 kDa caspase
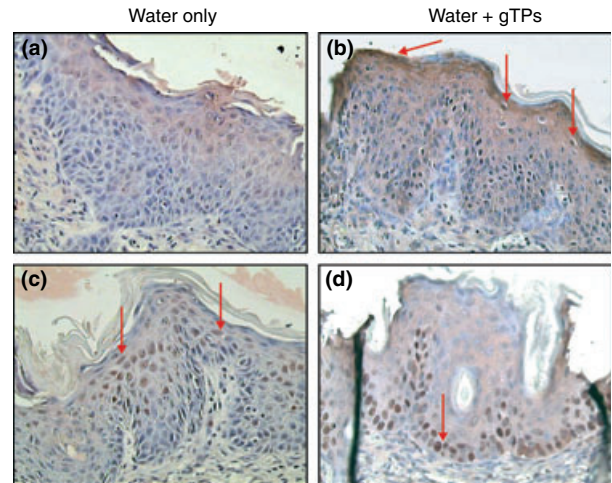


**Figure 3.** Immunostaining of skin samples from water-treated (a, c) and water-GTE-treated (b, d) flaky skin mice using caspase 14 antibody (a, b) or PCNA antibody (c, d). Nuclear caspase 14 staining appeared in the junction of the granular layer and the cornified layer in water-GTE-treated sample (arrows, b). Immunostaining for PCNA, a marker for cell proliferation, was localized primarily in the basal layer of the water-GTE-treated sample (arrow, d), but the water-treated sample exhibited staining in suprabasal layers (arrows, c). The epidermal portion of the samples was photographed at a 400× magnification.
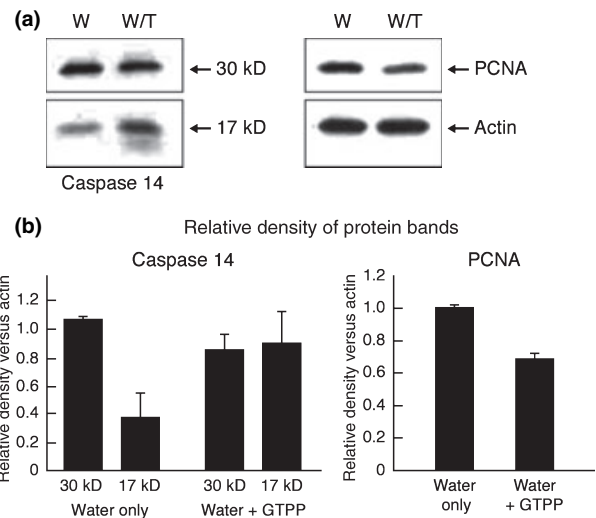


**Figure 4.** (a) Western blot analysis of homogenized skin samples (50 μg/lane) from a water-treated flaky skin mouse (W), or a water-GTE-treated flaky skin mouse (WT), using antibodies against caspase 14, PCNA and actin. More caspase 14 was processed from the 30 kDa procaspase 14 to the 17 kDa catalytically active subunit in the water-GTE-treated skin sample. Conversely, PCNA expression was greater in the water-treated skin sample. Actin served as an internal control for protein quantity. (b) Protein levels of caspase 14 (30 and 17 kDa) (left panel), and PCNA (right panel) determined by density measurements of the immunoreactive bands from three experiments.

14 (34), this result suggests that cornification is greater in the water-GTE-treated skin. The water-treated skin exhibited PCNA immunostaining in all epidermal layers (Fig. 3c,

arrows), while PCNA immunostaining was found primarily in the basal layer of the water-GTE-treated samples (Fig. 3d, arrow), as observed in normal skin. Results of the PCNA Western blot analysis of PCNA levels confirmed that PCNA levels were reduced in the water-GTE-treated skin samples (Fig. 4).

## Discussion

Only certain epithelial tissues associated with barriers express significant levels of caspase 14 (9,35). In the epidermis, caspase 14 is expressed in the suprabasal layers, and catalytically active caspase 14 (comprised of the 17 and 11 kDa subunits) is found in the cornified layers (34). We previously reported that EGCG-induced differentiation is associated with coordinated p57 and caspase 14 expression in NHEK (26). Results from the current study demonstrated that exponentially growing HNEK and cancer cells derived from oral epithelium (OSC2), salivary gland (HSG) and the epidermis (A431) do not express significant levels of caspase 14 (Fig. 1a). However, when NHEK undergo EGCG-induced differentiation, caspase 14 levels are elevated significantly (24,26, Fig. 1a). In contrast, OSC2, HSG and A431 cells, despite originating from epithelia, failed to respond to EGCG by elevating caspase 14 (Fig. 1a), or p57 (23,26). These observations suggest that EGCG induction of caspase 14 expression is associated with differentiation of normal epithelial cells, rather than a general property of epithelial-derived cells.

Although psoriasiform lesions, such as those in psoriasis, are considered to have an epithelial and an immunological component that interact with each other, evidence from a recent study shows that deletion of Jun proteins (a group of AP-1 transcription factors downstream from MAPK pathways) in the epidermis is sufficient to produce a psoriasiform skin disease in mice (28). The p38 MAPK pathway is essential for EGCG-induced expression of involucrin, a marker for NHEK differentiation (36). Results from the current study demonstrated that inhibition of p38 MAPK led to suppression of EGCG-induced expression of both caspase 14 and p57 in NHEK, while inhibition of JNK suppressed EGCG-induced expression of caspase 14, but not of p57 (Fig. 1b). Our results indicate that MAPK signalling pathways are essential for caspase 14 and p57 expression in NHEK cells, and suggest that the MAPK pathways are more broadly required for EGCG-induced NHEK differentiation (36). In human psoriatic epidermis, while caspase 14 is down-regulated (33), conflicting results showed that either both JNK and ERK are up-regulated, but not p38 (37), or both p38 and ERK are up-regulated, but not JNK (38). In conjunction with data presented here, this suggests that MAPK signalling pathways are necessary but insufficient to induce differentiation in psoriasiform lesions.

Our *in vitro* observations suggested that GTPs could have an effect on psoriasiform lesions. To investigate this possibility, we performed a small study using topical application of GTE containing 40% EGCG, in the flaky skin mouse model. The rationale for using GTE instead of purified GTPs is that GTE is naturally available without any chemical or compositional modification. The epidermis of the flaky skin mouse exhibits hyperproliferation, aberrant differentiation, and inflammation after the onset of symptoms (when they are 4–5 weeks old). The autosomal recessive mutation in these animals is referred to as the flaky skin mouse mutation, *fsn* (32), localized to the Tetratricopeptide repeat domain 7 (Ttc7) gene (39). Skin grafts from the *fsn/fsn* mouse to athymic nude mice maintain the psoriasiform phenotype, associated with elevated DNA synthesis (33). This indicates that epidermal keratinocytes are essential for the pathogenesis of the psoriasiform phenotype manifested by the *fsn* mutation, and that T-cell immunity is not significantly involved in the pathology of the flaky skin mouse, consistent with results from c-Jun and JunB knockout mice (28).

All water-treated (control) *fsn/fsn* animals exhibited visible skin lesions at 10 weeks of age, which became severe at week 11 (data not shown). Although the GTE-treated animals exhibited visible skin lesions at week 11.5 of age, they remained moderate until skin samples were collected on week 13. Histological analyses showed that the water-treated skin samples exhibited marked hyperkeratosis, mild parakeratosis, modest acanthosis, elongated rete ridges, and modest dermal inflammation; characteristics which are consistent with a psoriatic-like dermatitis. In contrast, GTE-treated skin samples exhibited slight hyperkeratosis, no parakeratosis, mild acanthosis, no elongated rete ridges, and mild dermal inflammation (Fig. 2). Consistent with *in vitro* NHEK cell culture studies, when the epidermis of skin samples from the two groups were immuno-stained for caspase 14, the GTE-treated epidermis (Fig. 3b) exhibited higher staining of caspase 14 in the stratum corneum than the water-treated epidermis (Fig. 3a). Further, the caspase 14 is predominantly located in the juncture between the stratum cornuem and the stratum granulosum in the epidermis of the GTE-treated animal (Fig. 3b). Similarly, PCNA protein expression in GTE-treated epidermis is localized predominantly in the stratum basalis (Fig. 3d), but PCNA staining was observed in all of the epidermal layers in the water-treated epidermis (Fig. 3c). These results were further confirmed by Western blot analyses using the skin samples. As shown in Fig. 4, GTE-treated skin possessed lower levels of the 30 kDa catalytically inactive caspase 14, but higher levels of catalytically active 17 kDa subunit than those of water-treated skin. The protein levels of PCNA were significantly reduced in the GTE-treated skin compared with the water-treated skin (Fig. 4). Collectively, these results suggest that topical application of 0.5%

GTE can reduce the severity of psoriasiform lesions in this animal model, both in terms of gross morphology, and at the level of expression and localization of caspase 14 and PCNA. This is consistent with the *in vitro* observations of the effects of EGCG on NHEK cells.

In conclusion, our data demonstrate that p38 and JNK MAPK pathways are essential for EGCG induction of p57 and caspase 14, a protein needed for cornification and barrier formation. In the flaky skin mouse epidermis, GTE restored, in part, the skin barrier by (i) reducing histological abnormalities (Fig. 2), (ii) promoting proteolytic processing of the 30 kDa caspase 14 into the active form, and (iii) modulating PCNA expression. These findings, to the best of our knowledge, have not been reported previously. Further investigation is needed to delineate fully the signal transduction pathways activated by EGCG to regulate gene expression leading to reduced inflammation, hyperproliferation, and hypodifferentiation. Our results also suggest that GTE, a non-toxic plant-derived extract, could be potentially useful in treating inflammatory skin conditions.

## Acknowledgements

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