SIG1191: A Novel Cosmetic Functional Ingredient with Anti-inflammatory Properties and Skin Hydrating Potential

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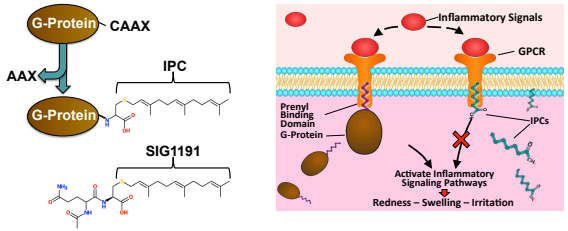
Abstract

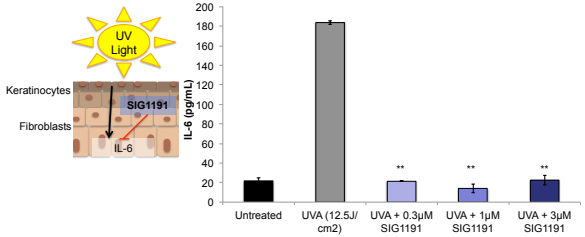
Isoprenylcysteine compounds that modulate the intracellular signal transduction activity of G-proteins have been shown to regulate the *in vitro* responses of inflammatory cells. Topical *in vivo* anti-inflammatory activity of the archetype of this class: N-acetyl-S-farnesylcysteine (AFC), was subsequently established leading to its use as a cosmetic ingredient in skin care products. We report here, a second generation IPC derivative of AFC, SIG1191 shows anti-inflammatory activity in the TPA-induced pro-inflammatory cytokine production in primary human keratinocytes. SIG1191 also demonstrates anti-inflammatory properties *in vitro* reducing UVA-induced cytokines in cultured dermal fibroblasts. Furthermore, SIG1191 inhibits inflammatory mediator COX-2 gene expression in cultured skin equivalents. In addition to its anti-inflammatory properties, we present here that SIG1191 potentially targets skin hydration and aging by modulating Aquaporin-3 (AQP3) expression. Aquaporin-3 (AQP3) and Aquaporin-9 (AQP9) regulate osmotic balance as integral pore forming cell membrane proteins facilitating water, urea and glycerol transport across cell membranes. They are both constitutively expressed by keratinocytes at the basal layer of the epidermis and are thus involved in regulating skin hydration. AQP3 expression levels have also been shown to decrease in response to both UVB exposure and skin aging. Here, we report SIG1191 dose-dependently stimulates AQP3 gene expression in both monolayer keratinocytes and 3D skin equivalent cultures. Results show that only AQP3 is dose-dependently increased by SIG1191, whereas AQP9 is not affected. Increase of AQP3 expression was independent of PPARγ activation. Altogether, these studies demonstrate SIG1191 is a novel cosmetic ingredient that can potentially provide skin hydration by increasing Aquaporin-3 and possesses anti-inflammatory properties to help protect the skin.

Fig 1. IPC Analogs: Structural Mimics of G-Protein C-Terminus



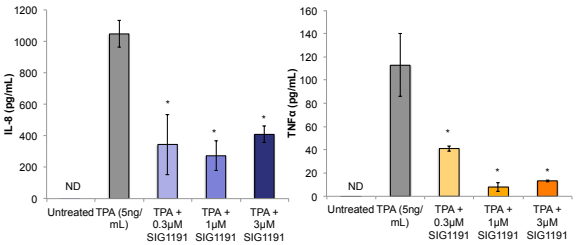
G-proteins participate in eliciting inflammatory responses such as the release of pro-inflammatory mediators and the migration and activation of inflammatory cells. Located near the end of each G-protein is a conserved cysteine residue modified with a prenyl tail (either 15 or 20 carbon side-chain). IPC analogs (eg. SIG1191) are structural mimics of the lipidated C-termini of the Gγ subunit of all heterotrimeric G-proteins, as well as all small molecular weight GTPases (e.g. Ras, Rho and Rac).

Fig 2. SIG1191 inhibits UV-induced Inflammatory response



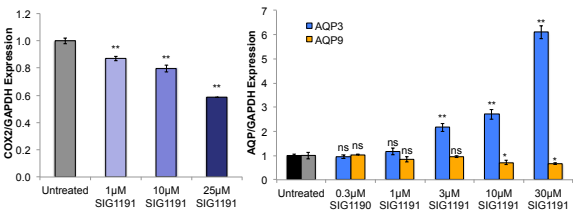
Primary Human Dermal Fibroblasts (HDFs) were seeded in 24-well plates and cultured for 24 hours at 37ºC and 5% CO_2 before treatments. Cells were cultured in the presence of each compound for 24 hours. Later, compounds were removed and cells were irradiated with 12.5J/cm^2 UVA. Media supernatants were collected after 24 hours and analyzed by ELISA for IL-6 protein levels. *p<0.01 by ANOVA test compared with UVA irradiated cells.

Fig 3. SIG1191 inhibits TPA-induced Inflammatory response



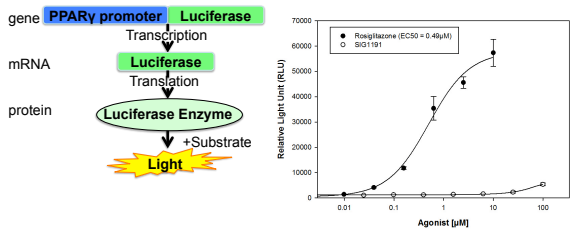
Normal Human Epidermal Keratinocytes (NHEKs) were seeded in 96-well plates and cultured for 24 hours at 37ºC and 5% CO_2 before treatments. Cells were pre-treated with compounds for 2 hours. Later, TPA (5ng/mL) was added with compounds and incubated for 6 hours. Cell toxicity was measured by MTS reduction assay and media supernatants were collected and analyzed by ELISA for IL-8 and TNFα. *p<0.05 by ANOVA test compared with TPA-only treated cells. (ND=non detected)

Fig 4. SIG1191 increases AQP3 expression



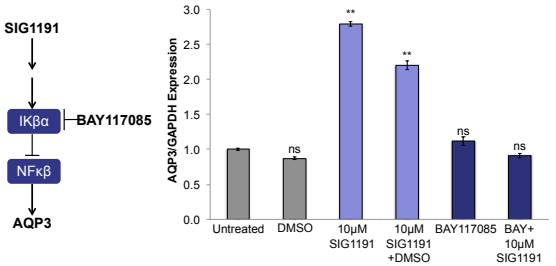
Normal Human EpiDermal Keratinocytes (NHEKs) were seeded in 6-well plates and cultured for 24 hours at 37ºC and 5% CO_2 before treatments. SIG1191 was treated in culture media and incubated for an additional 24 hours. Total RNA was extracted and performed qPCR for human Prostaglandin-endoperoxide synthase-2 (COX2), aquaporin-3 (AQP3), and aquaporin-9 (AQP9) using GAPDH gene as internal control. *p<0.05; **p≤0.01 by ANOVA test compared with untreated cells as control (ns = not significant).

Fig 6. SIG1191 increases AQP3 independent of PPARγ activation



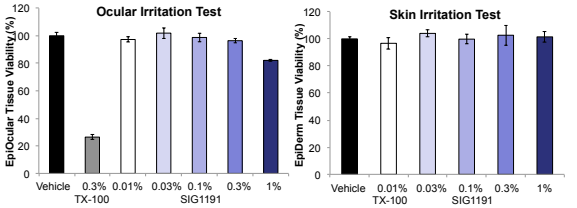
SIG1191 dose-response analysis of the human PPARγ (ligand-dependent transcription factor) activity was performed using luciferase reporter cells (Indigo Biosciences) and compared to rosiglitasone as reference agonist ligand.

Fig 7. SIG1191 increases AQP3 by NFκβ pathway



Normal Human EpiDermal Keratinocytes (NHEKs) were seeded in 6-well plates and cultured for 24 hours at 37ºC and 5% CO_2 before treatments. NFκβ pathway inhibitor (BAY117085) was co-treated with SIG1191 and incubated for an additional 24 hours. Total RNA was extracted and performed qPCR for human aquaporin-3 (AQP3) gene using GAPDH gene as internal control.

Fig 5. SIG1191 is a non irritant in 3D models



SIG1191 formulations were tested for skin and eye irritation in reconstructed human epidermis 3D models. EpiDerm™ and EpiOcular (MatTek®) tissues were acclimated for 1-24 hours and then treated topically with SIG1191 formulations (0.01-1%) and TritonX-100 (0.3% w/v), used as positive control. Tissue viability levels were measured by the MTT reduction assay 48 hours after treatments. The levels of tissue viability after each treatment were compared to vehicle group to estimate the potential for skin or ocular irritation. **p< 0.01 by ANOVA test compared with untreated tissues as control (ns = not significant).

Summary/Conclusions

- **SIG1191 demonstrates anti-inflammatory properties *in vitro* reducing UVA, UVB and TPA induced pro-inflammatory cytokine production in primary human keratinocytes and fibroblasts.**
- **SIG1191 potentially targets skin hydration and aging by modulating Aquaporin-3 (AQP3) expression in both monolayer keratinocytes and 3D skin equivalent cultures by the NFκβ pathway and independent of PPARγ activation.**
- **In conclusion, SIG1191 demonstrates to be a novel cosmetic ingredient that can potentially provide skin hydration by increasing Aquaporin-3 and possesses anti-inflammatory properties to help protect the skin.**