SUPPLEMENTARY MATERIALS

Supplementary 1: Cell Culturing Procedure

HCjE Cell Cultures

HCjE grew in 75 cm^2 tissue culture flasks in freshly made filter-sterilized KSFM, supplemented with Bovine Pituitary Extract (BPE, 25 µg/mL), Epidermal Growth Factor (EGF, 0.2 ng/mL), CaCl2 (0.3 mM) and Pen/Strep (100 units/mL). Once the growth reached 50% confluency, the cultures were kept in a maintenance medium till the end of assays. The maintenance medium contained a mixture of the complete KSFM, low calcium DMEM (GIBCO) and F12 (GIBCO) at a ratio of 2:1:1. The growth and maintenance of HCjE was done under a standard cell culture condition (37C, 5% CO2 and 100% relative humidity).

HCLE Cell Cultures

To prepare HCLE cultures for testing SKQ1 *in vitro*, a frozen stock of HCLE (at passages of 23^{rd}) was resurrected in a 75 cm^2 tissue culture flask in freshly made, filter-sterilized complete growth medium containing KSFM, supplemented with Bovine Pituitary Extract (BPE, 25 µg/mL), Epidermal Growth Factor (EGF, 0.2 ng/mL), CaCl2 (0.3 mM) and Pen/Strep (100 units/mL).

Preparation for Wound Healing Experiments

To initiate a wound healing experiment, the HCLE cultures (at passages between 26-36^{th}) were digested with 0.05% trypsin-EDTA (GIBCO) and re-distributed to grow in multiple 6-well cell culture plates until approximately 85% confluent. Two washes were performed using a pre-warmed, expired KSFM, followed by re-growth in a maintenance medium containing a mixture of the complete KSFM, low calcium DMEM (GIBCO) and F12

(GIBCO) at a ratio of 2:1: 1, supplemented with (for treated groups) or without (for control groups) 50 nM of SKQ1 for 1 hour before scratching.

Supplementary 2: MTT Assay Methodology

MTT Assay to Determine Cytotoxicity in HCjE Cells

Experiments included untreated cells (healthy cell control) as 100% viability, formalin–treated cells (dead cell control) as 0% viability and SKQ1 treated cells at various concentrations from 0.25 to 25,000 nM. Each group had 3 repeats and the entire experiment was repeated at least twice. Controls treated with the diluent-only medium were also conducted side-by-side at concentrations equivalent to the SKQ1-containing groups. At the end of each experiment, the absorbance values at A690 (background) were subtracted from absorbance values at A572 nM and then converted to cell viability. The resulting data was plotted against SKQ1 concentrations. The 50% cell toxicity value (TC50 Value) was calculated using a linear regression formula embedded in Excel.

MTT Assay to Evaluate HCLE Cell Proliferation

To evaluate if SKQ1 has a role in HCLE cell proliferation, extensively diluted single HCLE cells were inoculated into a 24-well plate in the growth and maintenance medium supplemented with various concentrations of SKQ1 ranging from 0 to 400 nM. Cell growth was tracked photographically at 24, 48 and 144 hours after inoculation. At the end of the experiments, a standard MTT assay was conducted, except the stained cells were dried and photographed prior the addition of acidic isopropanol to dissolve formazan and to measure the absorbance at A572 and 690 nm. Two independent such experiments were conducted, each had duplicate repeating wells.

Supplementary 3: Data Analysis

Analysis of Data from HCjE Inflammation Experiments

To normalize the data for easier interpretation of results, the following calculations were performed:

$$Degree\ of\ PGE2\ Production\ (with\ respect\ to\ IL1\beta) = \frac{\left(\frac{I_n}{I_0}\right)}{\left(\frac{C_n}{C_0}\right)}\ x\ 100\%$$

$$Degree\ of\ PGE2\ Production\ (with\ respect\ to\ TNF\alpha) = \frac{\left(\frac{T_n}{T_0}\right)}{\left(\frac{C_n}{C_0}\right)}\ x\ 100\%$$

Where:

C_0 = PGE-2 production at 0 nM concentration of SKQ1without IL-1β, or TNF-α sensitization;

C_n = PGE-2 production at *n* nM concentration of SKQ1 without IL-1β or TNF-α sensitization;

I_0 = PGE-2 production at 0 nM concentration of SKQ1with IL-1β sensitization;

I_n = PGE-2 production at *n* nM concentration of SKQ1 with IL-1β sensitization;

T_0 = PGE-2 production at 0 nM concentration of SKQ1with TNF-α sensitization;

T_n = PGE-2 production at *n* nM concentration of SKQ1with TNF-α sensitization.

Analysis of Data from HCLE Wound Healing Experiments

Rates of wound healing (%) of a specifically marked location at a particular time-point were calculated as follows:

$$Rate\ of\ wound\ healing(\%)$$

$$= [1 - \frac{(\text{area of treated wound at a time })}{(\text{area of original wound at time zero})}]x\ 100\%$$

To exclude any potential outliers within an experiment, the “Mean +/- 3SD” method was employed. Data was then calculated with MS Excel and presented as the mean +/- SD for all the measurements at a particular time-point, both for SKQ1-treated group and for untreated controls. Two (2) independent experiments with the same conditions were conducted, each had 2 repeating wells, and each well contained 3 spots to be monitored. Rates of SKQ1 treated over rates of untreated groups over the times of treatments were plotted. Two-tailed bi-variance Student’s *t*-test was run; significant differences between SKQ1-treated and un-treated groups were established when $*p<0.05$ or $**p<0.01$ or $***$ $p<0.001$.