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SUPPLEMENTARY MATERIALS AND METHODS

Human cutaneous SCC line (A431) cells

Human cutaneous SCC cell line A431, (Sigma Aldrich), cultured at 37 °C in a humidified 5% CO2 environment in DMEM supplemented with 2 mM L-glutamine, 4 g/L D-glucose, penicillin, streptomycin and 10% fetal bovine serum (FBS) (Gibco ThermoFisher).

Cell viability assay

50 µl of cells (A431 @ $1x10^5$ cells/ml) were seeded in 96 well-plates for 24 hours before atorvastatin [Sigma-Aldrich CAS 344423-98-9] or simvastatin [Cayman CAS 79902-6309] were added at increasing concentrations with supplementary volume of medium to a final volume of 100 µl, and incubated for 72 hours. 50 µl of XTT reagent solution (0.1 ml of activation solution to 5ml of XTT reagent) were add to each well, and incubated for 2 h. Cell viability was determined using ELISA reader at wavelength of 475 nm with background subtraction at 630 nm. Each experiment was performed in triplicate and repeated at least three times.

Apoptosis assay

A431 ($2.5x10^5$ cells/ml) were seeded in 30mm plates and incubated at 37 °C with 5% CO2 in supplemented DMEM for 24 hours before either atorvastatin or simvastatin were added at increasing concentrations and incubated for 48 hours, then twice washed in 3 ml phosphate buffered saline (PBS) and centrifuged @1300 rpm for 5 min at 21°C, washed in 0.3 ml 1× binding buffer, and centrifuged again (3000 rpm, 5 min). Then, cells were stained with annexin V-FITC (Abcam, UK) and PI. Cells were resuspended in 100 µl 1×binding buffer and 1 µl of annexin-FITC, incubated for 15min at room temperature (RT), protected from light. Cells were centrifuged (3000 rpm, 5 min, RT), washed with 0.2 ml 1× binding buffer and resuspended in 195 µl 1× binding buffer. 5 µl of PI were added just before the cells were transferred to FACS tubes each containing 300 µl PBS at RT, and samples were immediately analyzed by flow cytometer (FACS Calibur) using Kaluza 1.3 analysis software. Apoptotic cells included all annexin V positive cells (early and late apoptosis).

Hanging Drop (spheroid) assay

A431 were harvested and centrifuged to produce $2.5x10^5$ cells/ml suspensions in DMEM alone (Untreated), DMEM with 20µM or 40µM simvastatin and DMEM with 80µM or 160µM atorvastatin. Said 5 groups of cell suspension were seeded in discrete 20µl drops in 100mm plates, turned over onto a plate lid with 250µl of PBS and incubated at 37°C with 5% CO2 for 144 hours. Each study group was seeded at least 20 droplets per plate per incubation period and the experiment was repeated at least 3 times providing over 1200 droplets for analysis overall. The droplets were observed at the end of 144-hour incubation via Light microscope

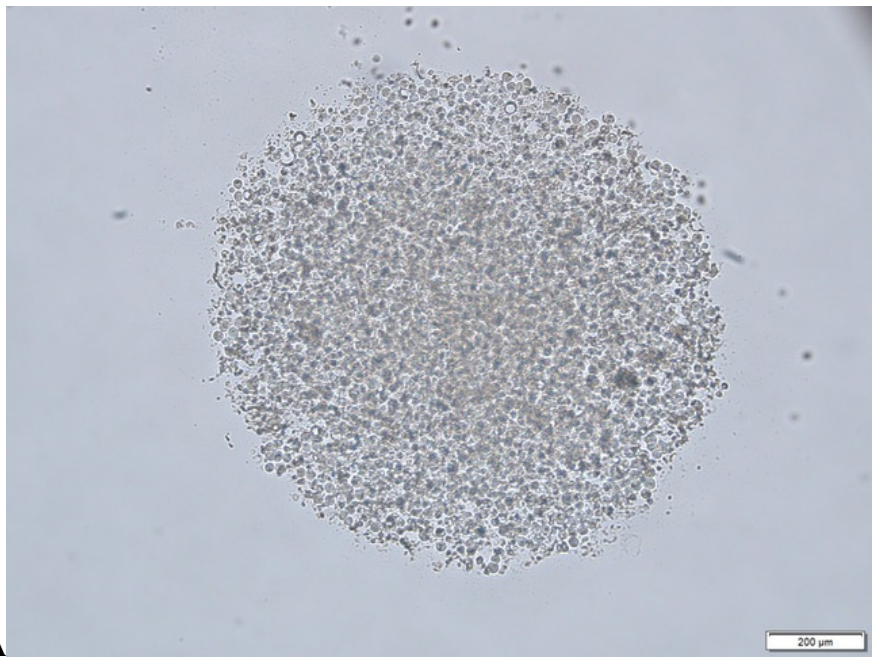
(Olympus BX52, Japan) assessed for the presence of a 3-dimensional spheroidal cellular structure (binary assessment) and an image for each droplet was digitally recorded (Fig. S1).

Statistical analysis

Data were analyzed using Prism software version 9 (GraphPad). Two-tailed T-test and repeated measures analysis of variance (ANOVA) were used to compare groups. Differences were considered to be significant when the P value was less than 5%.

Fig. S1. The hanging drop assay is a qualitative test for the interactions between cultured cells. A 20µl drop of cell culture medium is suspended upside down for 144 hours and then observed under light and fluorescent microscopy. A binary assessment is made whether a spheroid has formed (A) or not (B).

A



B