Online supplement

Immunohistochemistry

Sections were de-paraffinized by incubation in xylene (MP Biomedicals, Santa Ana, CA) for 10 minutes, then change fresh xylene and incubate another 10 minutes. Sections were rehydrated through graded alcohol (2x 100%, 95% and 70% ethanol) for 2 minutes each before being placed in distilled water for at least 2 minutes.

Sections were blocked with a peroxidase blocking agent (Dako) for 2X15 minutes in a humid box at 37°C, then sections were washed with tris buffered saline – 0.05% Tween 20 buffer (TBS-T) (Ph 7.4) for 3X5 minutes, and blocked with non-immune blocking serum (Dako) for 30 minutes in a humid box at 37°C.

Sections were incubated with rabbit polyclonal anti-human collagen VIII alpha 1 antibody (Abcam) (1μg/ml), and performed in parallel with isotype control rabbit IgG antibody (Dako) (1μg/ml) overnight in a humid box at 4°C.

Sections were washed with TBS-T for 5X3 minutes followed by incubation with conjugated secondary antibody, labelled polymer anti-rabbit (EnVision+System-HRP) (Dako) for 45 minutes in a humid box at 37°C. Sections were washed with TBS-T for 5X3 minutes, and the tissue staining was visualized with substrate chromogen, liquid 3,3'-diaminobenzidine (DAB) (Dako) for 10 minutes at room temperature.

Sections were counterstained with Mayer's hematoxylin (Sigma Aldrich) for 5 minutes. Sections were then coverslipped by using organic mounting media DPX (Asia Pacific Specialty Chemicals, Australia).

Sections were inmaged on an Olympus BX60 microscope (Olympus, Hamburg, Germany) with manual light exposure and 'one push' white balance on a background region. Images were taken using an attached DP71 camera (Olympus). 10 images of immunostaining on each section were taken at 20X magnification. The positive staining area (the number of brown pixels in the image) on these images was quantified by using Fiji software (ImageJ).

Cell attachment assay

96-well culture plates (BD Biosciences, North Ryde, Australia) were exposed to growth medium for 72 hours and to quiescing medium for 24 hours then exposed to quiescing medium with different concentration of CSE (0.05% to 10%) for 72 hours. The 96-well culture plates were washed with PBS for 3 times.

Human ASM cells were seeded on these treated plates at a density of $5x10^4/cm^2$ in quiescing medium for 2 hours at 37°C/ 5% CO_2. Discard media and wash with PBS for 3 times to remove any unattached cells, and use 4% paraformaldehyde solution to fix the attached cells for 10 minutes. Attached cells were stained with 0.5% toluidine blue solution for 5 minutes then wash with distilled water for 3 times, and add 1%SDS solution and leave to solubilise at room temperature for more than 2 hours. The relative number of attached cells was measured by using spectrophotometry (Spectramax M2, Molecular Devices, Sunnyvale, CA, USA) at an absorbance of 595nm.

Wound healing assay

Oris cell migration assembly kit (Platypus Technologies, Madison, WI, USA) was used to perform wound healing assay. The black 96-well plate was exposed in growth medium for 72 hours and in quiescing medium for 24 hours then exposed in quiescing medium with different concentration of CSE (0.05% to 10%) for 72 hours. The black 96-well plate was washed with PBS for 3 times.

To create the wound on the treated black plate by using cell seeding stoppers according to the manufacturer's instructions. Briefly, insert stoppers into each well of the treated black plate, the stoppers will create a circle wound in the middle of each well. Apply the detection

mask to the bottom of the plate, the apertures on the mask will march the stopper circles on the wells.

The human ASM cells were labelled with cell tracker green (CMFDA) (Invitrogen) according to the manufacturer's instructions. Briefly, incubate human ASM cells in the cell tracker working solution (10μm) at 37°C in the dark for 30 minutes. Centrifuge the labelled cell suspension, discard the supernatant and keep the cell platelet.

The labelled human ASM cells were seed on the wounded black plates at a density of $5x10^4/cm_2$ in growth medium, and incubated at 37°C/ 5% CO_2 in the dark. After adhesion for 24 hours the stoppers were removed. Wash wells with growth medium twice and refill with growth medium (100μl/well), and continue incubate for 4 hours at 37°C/ 5% CO_2 in the dark.

The wound healing value was measured by using a fluorescence plate reader (Wallac VICTOR^2, Perkin Elmer, Waltham, MA, USA) read from bottom with setting excitation wavelength 485nm and emission wavelength 535nm.